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EFFECT OF COMBINED STERILIZATION ON THE STRUCTURAL AND FUNCTIONAL PROPERTIES OF BONE IMPLANTS: A STUDY OF RADIATION-INDUCED CHANGES IN COLLAGEN

This paper presents a comprehensive analysis of the effects of radiation and combined exposure on the structure and properties of bone tissue, with a particular emphasis on changes in the collagen matrix. This study aims to optimize a combined sterilization technology for biological implants and bone tissue, ensuring effective inactivation of pathogenic microflora while maintaining the structural integrity and biomechanical properties of the material. Particular attention is paid to the effect of ionizing radiation on the intermolecular interactions of collagen, its spatial organization, and degradation processes. A mathematical model has been developed describing changes in interfibrillar distances in collagen, enabling a quantitative relationship between the radiation dose and the level of protein matrix degradation. This work has practical implications for improving sterilization methods for biomaterials intended for transplantology and reconstructive surgery. The studies were conducted using Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), atomic force microscopy (AFM), and micromechanical analysis methods. For sterilization, an ozone-oxygen mixture obtained using a medical ozonizer with an adjustable concentration of active oxygen species was used. Radiation irradiation was carried out on a UELR-1-25-T-001 continuous-flow linear electron accelerator (Institute of Nuclear Physics, Lomonosov Moscow State University). It was found that combined sterilization allows reducing the radiation dose to 12 kGy, while maintaining the structural integrity of collagen and the mechanical properties of bone material. A model of collagen degradation under the influence of radiation is proposed, linking the dose load with a change in intermolecular distances; a quantitative correlation between the parameters Δr (according to the model) and structural changes in collagen was established; The possibility of reducing the radiation dose while maintaining the sterilizing effect through ozone pretreatment has been experimentally demonstrated. The proposed combined sterilization technology provides a pronounced synergistic effect, enabling a high level of sterility while preserving the biophysical properties of bone implants. This approach significantly reduces the radiation dose and thermal impact compared to traditional radiation sterilization methods, ensuring safer use for biomedical purposes.

Keywords: radiation sterilization, ozone, collagen, bone tissue, FTIR, atomic force microscopy, mathematical modeling

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Introduction. Modern trends in reconstructive surgery and tissue engineering place increased demands on the quality and biocompatibility of bone implants. One of the key conditions for their safe use is effective sterilization with minimal changes in the material structure. Radiation sterilization has proven itself as a highly effective method, ensuring reliable inactivation of pathogenic microorganisms without the use of toxic reagents. However, excessive radiation doses (more than 20–25 kGy) lead to the degradation of the organic bone matrix, particularly collagen, which significantly reduces the mechanical strength and biological activity of the material [2, 3, 7, 9].

Collagen is the main structural protein of connective tissue, providing strength, elasticity, and biocompatibility to bone [4, 11]. Its molecular organization is sensitive to physicochemical influences, especially ionizing radiation. Damage to the collagen matrix manifests itself as cleavage of peptide bonds, destabilization of the secondary structure, and an increase in intermolecular distances between fibrils, which leads to disruption of the bone tissue architecture. Understanding the mechanisms of radiation-induced collagen degradation is key to the development of gentle sterilization regimens. One promising area for optimizing radiation sterilization is the use of combined technologies that include preliminary exposure to ozone [8]. Ozone has pronounced bactericidal properties and promotes partial destruction of the cell membranes of microorganisms, increasing the effectiveness of subsequent radiation exposure. This approach allows for a reduction in the required radiation dose, thereby limiting radiation-induced damage to structural proteins. It has previously been shown that radiation causes dose-dependent changes in the spectra of collagen amide groups (Amide I, II, III) in the 1670–1500 cm^{-1} range, indicating disruption of the spatial organization of the protein matrix [8]. However, the quantitative patterns of changes in intermolecular distances and collagen fibril morphology parameters under the influence of radiation have been insufficiently studied. To address this issue, a mathematical model was developed in this study that relates the radiation dose to changes in the distances between dipoles in the collagen structure. Ozone treatment prior to radiation exposure not only enhances the sterilization effect but also stabilizes the structure of the protein matrix. This is due to an increase in oxygen content in bone tissue, which enhances the "oxygen effect" and reduces the radiation dose required to inactivate pathogens [8].

This study presents a comprehensive study of bone tissue collagen structures after various types of sterilization—se-

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lective (ozone and radiation) and combined. The primary objective is to assess the degree of radiation-induced collagen degradation and determine the optimal parameters for combined sterilization that ensure the preservation of bone tissue morphology and mechanical properties.

Materials and Methods. To study the structural changes in collagen in bone tissue exposed to ionizing radiation and ozone treatment, a combination of modern instrumental and computational methods was used. This allowed for a comprehensive characterization of the morphological, chemical, and mechanical changes in bone samples at the micro- and nanoscale levels. Compact fragments of bovine bone tissue (2 × 2 × 3 mm) were used as model samples. They were pre-cleaned, defatted, and dried at a temperature not exceeding 40 °C to preserve the collagen structure. Bone was considered as a two-phase system: a mineral component (calcium hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$) and an organic matrix, represented mainly by type I collagen, responsible for elasticity and plasticity. All samples were stored in sealed bags at room temperature until analysis. IR spectra were recorded on a Varian 7000 FTIR spectrometer in the 4000–400 cm^{-1} range with a resolution of 2 cm^{-1}. FTIR provided quantitative data on the state of the organic and mineral phases of bone tissue. The spectra were analyzed using the main characteristic absorption bands. For quantitative analysis, deconvolution of the amide bands (I and II) was used, which allowed us to assess the degree of collagen fiber degradation and changes in their cross-links under radiation exposure. The morphology and nanostructure of the collagen fibril surface were studied using atomic force microscopy on a Solver NEXT system (NT-MDT, Russia). Three-dimensional images of 5×5 μm and 10×10 μm areas were recorded with a vertical resolution of up to 1 nm. Using specialized software (Nova and Image Analysis 3.4), the following were calculated: average fibril thickness (d_o), interfibrillar distance (r), orientation parameter (θ), root-mean-square roughness (R_a), and structural ordering coefficient (S). Particular attention was paid to the change in r, the average interfibril distance, reflecting the degree of radiation-induced destruction of the collagen network. Surface morphological analysis and elemental composition were performed on a JEOL JSM-7800F microscope (Japan) equipped with an energy-dispersive analysis system. SEM made it possible to evaluate: surface topography (cracks, defects, porosity), element distribution (C, O, Ca, etc.), etc. Microhardness was determined by the Vickers method using a DM8 device (Italy). For each sample, measurements were performed, followed by statistical averaging, and the results were compared between groups (control, ozone, radiation, and ozone + radiation). To quantitatively describe radiation-induced changes in collagen structure, a physical and mathematical model was developed based on the dipole representation of the collagen molecule. The model relates the absorbed radiation dose (D) to the change in intermolecular distance (Δr) between the dipole centers:

$$E_{abs} = D \cdot m$$

$$U = -\frac{A}{r^6},\ A = \frac{2}{3}\frac{p^4}{kT}$$

where: E_{abs} is the absorbed energy, m is the mass of the sample, A is the dipole interaction constant, p is the dipole moment of the collagen molecule, k is the Boltzmann constant, T is the temperature (K). From this follows the relationship:

$$r_2 = \sqrt[6]{\frac{A}{\frac{A}{r_1^6} - \frac{E_{погл} \cdot c}{2N}}}$$

where: r_1 and r_2 are the distances between the dipoles before and after irradiation; N is the number of collagen molecules; c is a parameter that takes into account the radiation quality factor and the dipole constant.

The difference $\Delta r = r_2 - r_1$ characterizes the degree of radiation-induced collagen destruction. The calculated Δr values were compared with experimental data, which allowed for quantitative confirmation of the correlation between the radiation dose and changes in collagen structure. Microbiological tests were conducted to confirm the sterilizing effect of the combined technology. The highest efficiency in inactivating fungal and bacterial spores was observed with combined treatment with ozone and radiation at a dose of 12–15 kGy. Experimental data were processed using OriginPro 2023, Statistica 13.3, and Matlab. Student's t-test was used to compare results at a significance level of $p < 0.05$.

Results and Discussion. Experimental data confirm that exposure of bone tissue to ionizing radiation causes a series of interrelated changes in the organic (collagen) and inorganic (mineral) phases. At doses up to 10–12 kGy, relative structural stability is maintained; however, above 15–20 kGy, signs of collagen network degradation are observed, accompanied by a decrease in the intensity of amide bands in the IR spectra and an increase in microporosity in AFM and SEM images. Radiation initiates the formation of reactive radicals, leading to the cleavage of peptide bonds and the destruction of the collagen triple helix. This is manifested by changes in fiber configuration, an increase in interfibrillar distances, and a localized decrease in surface microhardness. Ozone treatment prior to irradiation plays a significant role, reducing the number of radiation-active centers and partially offsetting the energetic effects of gamma quanta or electron beams.

Infrared spectroscopy: changes in collagen structure. Table 1 shows the characteristic spectra of bone tissue before and after irradiation with doses of 12 and 20 kGy: 1) the amide I band (1670–1650 cm^{-1}), corresponding to stretching of the C=O bond in the peptide group, gradually decreases with increasing dose, indicating the destruction of the secondary structure (α-helix and β-sheets); 2) the amide II band (1550 cm^{-1}), reflecting combined C–N and N–H vibrations, becomes less pronounced at doses above 15 kGy, indicating partial destruction of hydrogen bonds in the protein; 3) a decrease in the intensity of the amide III band (1240 cm^{-1}), associated with collagen cross-links, is observed. 4) A relative increase in the intensity of phosphate bands (560 and 604 cm^{-1}) is observed in the mineral phase, which is associated with a partial loss of the organic component and a relative increase in the proportion of apatite. These results confirm that the collagen component of bone tissue is the most radiosensitive component. A decrease in the intensity of amide group bands at doses ≥20 kGy indicates degradation of the protein matrix, which correlates with a decrease in microhardness and a change in the surface nanorelief.

Atomic force microscopy: collagen nanomorphology. AFM studies revealed dose-dependent changes in the nanostructure of the collagen network. Control samples exhibited clearly defined fibril ordering with a characteristic transverse striation pitch of ~67 nm, which corresponds to the normal D-periodicity of type I collagen. After irradiation with a dose of 12 kGy, the structure retains a regular pattern, with only minor height fluctuations observed (Fig. 1a, Fig. 2a). However, at 20–25 kGy (Fig. 1b, Fig. 2b), a loss of order, fibril rupture, and

Table 1

Changes in amide and mineral bands at different dose levels

Stripe (cm^{-1})	Structure	12 kGy	20 kGy	Interpretation
1673 (amide I)	C=O (peptide)	Minor decrease (~5%)	Significant weakening (~20%)	Rupture of collagen helices
1550 (amide II)	C–N, N–H	Minor fluctuations	Decrease in intensity	Hydrogen bond disruption
1240 (amide III)	C–N + N–H	It is saved	A decrease of 15–18%	Loss of cross-links
1030 (PO_4^{3-})	Mineral grid	Minor increase	Relative share growth	Relative exposure of apatite
870 (CO_3^{2-})	Substitution carbonate	No changes	Minor decrease	Loss of part of the carbonate group

aggregate formation are observed. The measured nanostructure parameters are presented in Table 2. An increase in interfibrillar distances (r) and a decrease in order (S) are direct markers of radiation-induced changes. These data are in good agreement with the predictions of the theoretical model, which describes an increase in Δr with increasing dose (Table 3). The results of mechanical tests demonstrate that at doses ≥20 kGy, collagen destruction occurs, leading to weakening of interfibrillar bonds and a decrease in elasticity.

Comparison of experimental data and the model. The results of atomic force microscopy and spectroscopy were compared with model calculations of changes in intermolecular distances (Δr) with increasing dose (Table 3). The theoretically calculated increase in Δr correlates with the increase in interfibril distances observed experimentally (correlation coefficient $R^2 = 0.94$). This high convergence indicates that the model correctly describes the physical mech-

Table 2

Measured nanostructure parameters

Parameter	Control	12 kGy	20 kGy	25 kGy
Average fibril thickness d_o , nm	95 ± 8	97 ± 10	112 ± 12	130 ± 14
Interfibrillar distance r , nm	41 ± 5	43 ± 6	62 ± 9	75 ± 11
Ordering coefficient S	0.92	0.89	0.71	0.64
R_a (roughness), nm	18 ± 3	19 ± 3	27 ± 4	34 ± 5

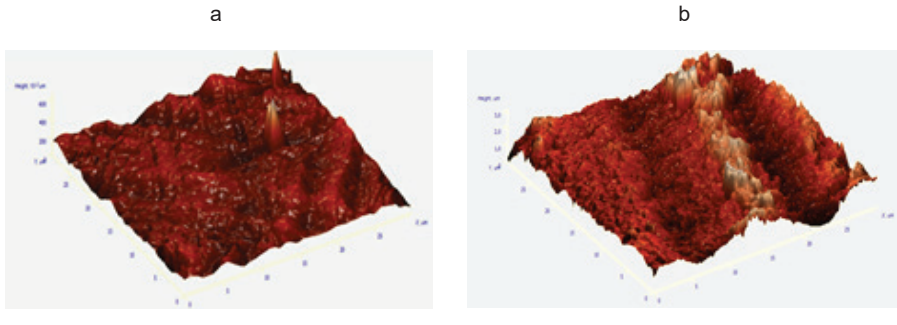

Fig. 1. Example of AFM images of collagen fibril structures after radiation exposure: a) 12 kGy, b) 20 kGy

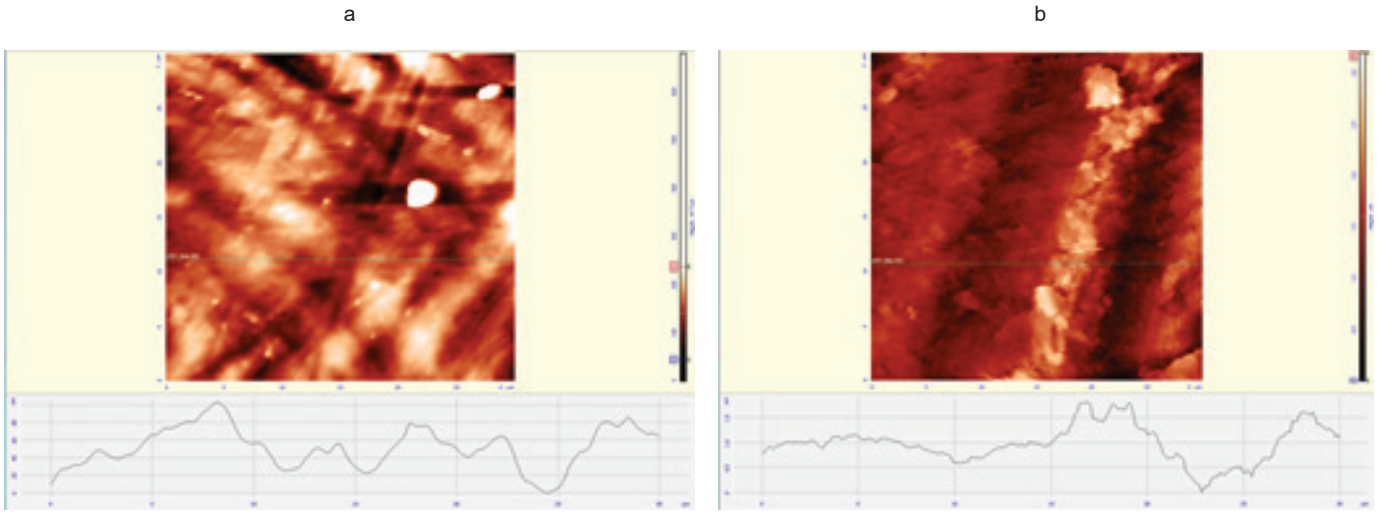

Fig. 2. Example of obtained images of the surface relief of samples (AFM) after exposure to radiation with a dose of: a) 12 kGy, b) 20 kGy

Table 3

Dependence of the change in intermolecular distance Δr on the values of the absorbed dose

Dose (kGy)	Δr (model), nm	Δr (AFM), nm	Deviation, %
5	4.6	5.0 ± 1.2	8.6
10	9.9	10.3 ± 1.5	4.0
12	12.3	12.8 ± 1.6	3.9
20	23.3	24.1 ± 2.2	3.4
25	31.2	30.8 ± 2.9	1.3

anism: radiation leads to an increase in the distances between collagen dipoles and a weakening of intermolecular interactions, resulting in a loss of order and a decrease in strength.

Experimental data and mathematical modeling mutually confirm that increasing the radiation dose leads to destruction of the protein matrix, manifested by an increase in intermolecular distances, cleavage of peptide bonds, and a change in the topology of the fibrillar structure. These effects are evident at doses of 15–20 kGy and become pronounced above 25 kGy, accompanied by a decrease in microhardness and a disruption of the elastic properties of the bone surface. IR spectroscopy revealed a decrease in the intensity of amide bands I–III, while AFM and SEM confirmed morphological changes such as fragmentation, thickening, and fibril aggregation. The developed model of collagen intermolecular interactions under radiation exposure agrees well with experimental data: an increase in the absorbed dose leads to an increase in the distance between dipoles (Δr), which can be considered a quantitative criterion for the degree of protein matrix degradation. The identified synergistic effect of ozone pretreatment is of particular significance. Ozone reduces the concentration of microbial cells and their radioresistance. This allows subsequent radiation exposure to be reduced to doses of 11–12 kGy, which ensures sample sterility without noticeable changes in the morphological and mechanical properties of the bone. This makes the combined technology a promising alternative to standard methods of bone implant sterilization, which use doses of 25 kGy or higher.

Conclusion. The obtained results open up the possibility of creating gentle technologies for radiation sterilization of bone and collagen-containing biomaterials, applicable not only in clinical practice (manufacture of bone implants), but also in paleontological research, where preservation of the structure of ancient bone remains is required without their thermal or chemical destruction [1, 5, 6]. The developed technology can be applied: in the production of bone implants and grafts in tissue banks; in sterilization of paleontological samples requiring preservation of micro- and nanostructure; in research laboratories to optimize the radiation-chemical stability of protein materials; for the development of new biocompatible composites based on collagen, etc. In addition, the mathematical model proposed in the work can be used to predict the degree of structural changes with various types of ionizing radiation (gamma, electron beam, X-ray). Prospects for further research: expansion of the range of dose loads, taking into account the relaxation time of collagen structures after irradiation; Using three-dimensional data correlation to construct spatial models of collagen network damage; and assessing the biocompatibility and osteoinductive properties of samples in cellular tests.

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References

1. Brown S., Higham C., Slon V. [et al.] Identification of a new hominin bone from Denisova Cave, Siberia using collagen fingerprinting and mitochondrial DNA analysis. Scientific Reports. 2016; 6(1):23559.

2. Cho W.T., Kim S.Y., Jung S.I. [et al.] Effects of Gamma Radiation-Induced Crosslinking of Collagen Type I Coated Dental Titanium Implants on Osseointegration and Bone Regeneration. Materials (Basel). 2021; 14(12):3268.

3. Crocker D.B., Akkus O., Oest M.E. [et al.] The influence of radiation-induced collagen chain fragmentation, crosslinking, and sequential irradiation on the high-cycle fatigue life of human cortical bone. Journal of the Mechanical Behavior of Biomedical Materials. 2024; 160:106759.

4. Fan L., Ren Y., Emmert S. [et al.] The Use of Collagen-Based Materials in Bone Tissue Engineering. International Journal of Molecular Sciences. 2023; 24(4):3744.

5. Kawahara K. [et al.] Identification of animal species by the MALDI-MS of collagen in animal glues of Chinese ink sticks. Proceedings of the 59th ASMS Conference and Allied Topics, Denver, CO. 2011.

6. Nakazawa T. [et al.] Mass Spectrometry of Collagen Preserved in Neolithic Animal Bones for the Identification of Species. Proceedings of the 66th ASMS Conference and Allied Topics, San Diego, CA. 2018.

7. Ng K., Allam N., Neshatian M. [et al.] Effects of Ionizing Radiation on the Biophysical Properties of Type I Collagen Fibrils. PLOS ONE. 2025; 20(4):e0319777. https://doi.org/10.1371/journal.pone.0319777

8. Nikolaeva N.A., Rozanov V.V., Chernyaev A.P. [et al.] The influence of combined sterilization factors on the structural and functional characteristics of bone implants. International Journal of Molecular Sciences. 2023; 24(19):14426.

9. Pendleton M.M., Emerzian S.R., Liu J. [et al.] Effects of ex vivo ionizing radiation on collagen structure and whole-bone mechanical properties of mouse vertebrae. Bone. 2019; 128:115043.

10. Rozanov V.V., Matveichuk I.V., Chernyaev A.P. [et al.] Changes in the Morphological and Mechanical Characteristics of Bone Implants upon Radiation Sterilization. Bulletin of the Russian Academy of Sciences: Physics. 2019; 83(10):1311–1315.

11. Rou L., Shiqing X., Yanning G. [et al.] Application of collagen in bone regeneration. Journal of Orthopaedic Translation. 2025; 50:129–143.