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New Composite Materials with Cross-Linked Structures Based on Grafted Copolymers of Acrylates on Cod Collagen

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Abstract: This article presents new composite materials with cross-linked structures based on grafted copolymers of collagen with acrylates, which were prepared under photocatalytic conditions using the complex oxide $RbTe_{1.5}W_{0.5}O_6$ and additional modifiers. Cross-linking agents for acrylate fragments were used. Triethylene glycol dimethacrylate (TEDMA) was used in small volumes, and acrylic acid (AA) was used in an amount similar to the MMA concentration. The properties of the obtained graft copolymers were studied. Thus, collagen containing acrylate graft copolymers with cross-linked structures, which are highly soluble in water, was obtained. Addition of the protein substrate modifier (polyethylene glycol (PEG)) in an amount comparable to collagen resulted in a coagulate with a cross-linked structure. The gel easily released water during drying in vacuum and formed a polymer composite film. According to the results, the new polymer materials which were obtained were fungi-resistant. Due to observed properties, the obtained polymer samples could be considered as new materials for medical products, including colloidal solutions, gels, films.

Keywords: cod collagen; PMMA; graft copolymerization; complex oxide $RbTe_{1.5}W_{0.5}O_6$; cross-linked structure; modification; fungus resistance

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1. Introduction

Environmental problems, which are the result of rapid technological development, concern everyone. The most important problems among them include waste accumulation and its impact on the environment. Therefore, the creation of a new generation of materials with the required operational characteristics, including high reliability, is strongly linked with the use of new approaches in synthesis. Nowadays, the principles of "green chemistry" are priority areas in chemistry. Photocatalytic materials based on metal oxides are environmentally friendly alternatives to chemical sources of active particles in reactions. They allow us to avoid excipients during synthesis, to reduce the number of stages in the process, etc. The widely studied photocatalytic processes are water splitting with hydrogen formation, decomposition of organic compounds, and polymerization transformations. The photocatalytic preparation of biomedical materials based on natural polymers is particularly attractive. Modern polymer materials, which are capable of biodegradation after usage, have less impact on the environment, which is economically beneficial. Such research represents a new stage in the development of medical technologies that have emerged at the intersection of medicine, biology, physics, chemistry, etc.

The development of new biodegradable materials requires active research in the field of polymer chemistry; thus, hybrid materials based on natural and synthetic polymers have been widely studied in the literature [1–6]. The most often used materials for this purpose are collagen- or gelatin-based substances [6–9]. Previously, in our studies, grafted copolymers PMMA-CC have been obtained by photocatalysis using complex oxide $RbTe_{1.5}W_{0.5}O_6$

and characterized as promising reagents in green chemistry [10–15]. The composition and structure analysis of these copolymers indicates the prospects for their use as precursors for the production of biodegradable materials. In particular, it has been shown that grafted methyl methacrylate copolymers with collagen undergo enzymatic hydrolysis, but at a slightly lower rate than collagen [14]. Moreover, the most important characteristic of such a copolymer is resistance to fungi [16], which leads to the production of fungi-resistant coatings. However, grafted MMA copolymers on collagen can only be considered only as precursors of biodegradable materials. The creation of special-purpose materials requires the development of composites by introducing additional components with certain characteristics [17–35]. Thus, the research on the creation of composite materials with the inclusion of modifiers is significant and promising.

PEG is the most well-known and widespread modifier of the protein properties of substrates in medical and pharmaceutical practice [17–20]. The Society for Nutrition and Medicines of the USA (FDA) allows the use of PEG as a substance in medicine (production of medicines), food, and cosmetology [17]; it is also used as a food additive (E1521) in the EU and the Russian Federation [36]. The modification of proteins using PEG (pegylation) leads to changes in the properties of the peptide substrate. For example, covalent bonds of PEG with fragments of the amino acids lysine and arginine (carboxyl groups) prevent the cleavage of modified molecules during proteolysis [22]. Covalent coupling with protein often occurs with the nitrogen in the lysine amino group or with the histidine imidazole group. Moreover, such bonds activate the PEG hydroxyl groups. In addition, materials modified by PEG can form hydrogen bonds due to the hydrogen atoms of the main PEG chain, significantly increasing its hydrodynamic radius. Other modifiers besides PEG are also used.

Along with PEG, other modifiers are also used. Natural collagen (gelatin) is characterized by low chemical and mechanical stability, so, when "cross-linked" with various chemical agents or physical exposure, its strength and elasticity are usually increased [23–34]. Moreover, "crosslinking" is possible with the formation of covalent [8,20,23,25–30,32–35] and ionic [8,19,24,26,30] connections.

Changing the properties of natural polymers by adding chemical agents and modifiers is promising for the creation of new materials. Thus, the aim of this work was to obtain a composite material of a cross-linked structure based on grafted copolymer MMA and collagen. The synthesis was carried out under photocatalytic conditions, with complex oxide $RbTe_{1.5}W_{0.5}O_6$ modifying the reaction mixture with additives at the start and at the isolation stage. Thus, the following investigations were been carried out.

Adding PEG, which is the property modifier of protein substrates, allowed us to control the composition and property changes of the resulting composite in comparison to the PMMA-CC copolymer. Adding a cross-linking agent for acrylate formulations (TEDMA and AA) in the initial reaction mixture led to the formation of an additional ionic "crosslinking" in the composite after neutralization of the final product. A comparison of the composition and properties of the resulting composite and the PMMA-CC copolymer was presented. Neutralizing the solution of the final product allowed us to obtain coagulate in the mixture. An analysis of the fungus-resistant properties of the new composite material was performed.

2. Materials and Methods

2.1. Materials

Commercial reagents were used: acetic acid (analytical grade, Lega, Dzerzhinsk, Russia), sodium hydroxide (pure for analysis, Reahim, Moscow, Russia), acrylic acid (pure for analysis, Sigma Aldrich, Burlington, MA, USA), triethylene glycol dimethacrylate (Himtranzit, Dzerzhinsk, Russia), and polyethylene glycol (M_w = 4000, Norkem, Dzerzhinsk, Russia). A monomer, methyl methacrylate (pure for analysis, Sigma Aldrich, USA), was used; it was purified from the stabilizer by sequential washing with sodium hydroxide solution and with cold water until reaching a neutral pH. Then, it was dried using calcium

chloride and distilled in a vacuum (1.33 Pa) at 40 °C. The complex oxide $RbTe_{1.5}W_{0.5}O_6$ was obtained by the solid-state method, as described previously [37].

2.2. Isolation of Cod Collagen

Collagen was isolated according to the method described in [38] by extraction with acetic acid for one day at room temperature. The resulting acetic acid dispersion was dried to a constant weight under vacuum (1.33 Pa) at 50 °C.

2.3. Synthesis of Graft Copolymers under Photocatalysis Conditions

The emulsion was prepared by mixing 10% solution of CC and PEG with MMA, AA, and TEDMA at various ratios. The complex oxide was added into the ratio emulsion: catalyst = 180:1. The mixture was bubbled for 15 min with argon, then stirred (600 rpm) and irradiated with a visible light LED lamp (LED, 30 W) in the argon current for 5 h. After polymerization, the reaction mixture was centrifuged at 4000 rpm to separate the catalyst. Toluene was added to the emulsion for the extraction of the organic phase; then, the aqueous and organic phases were separated and analyzed. The dried aqueous phase was washed with chloroform using a Soxlet extractor at 61 °C for 10 h.

All compositions of the obtained samples are presented in Table 1.

Table 1. Compositions of the obtained copolymers.

Sample	Content	Ratio of Components, wt.%	Grafting, %
CCC-1	CC:MMA:TEDMA:AA:water	4.35:4.35:0.03:4.34:86.95	29
CCC-2	CC:MMA:TEDMA:AA:water	8.00:6.00:0.05:6.00:79.95	26
CCC-3	CC:MMA:TEDMA:AA:PEG:water	7.7:3.80:0.05:3.80:7.7:76.88	61

2.4. Analysis of Molecular Weight Characteristics by GPC

Molecular mass characteristics were determined by gel-penetrating chromatography (GPC). Aqueous solutions were analyzed using a high-performance liquid chromatograph, manufactured by Shimadzu CTO 20A/20A C (Japan), using LC solutions. Separation was performed using a Tosoh Bioscience TSKgel g3000swxl column with a pore diameter of 5 microns and a low-temperature light scattering detector (ELSD-LT II). The eluent consisted of 0.5 M acetic acid solution, the flow rate was 0.8 mL/min, and narrowly dispersed dextran standards with a molecular weight range (MW) of 1–410 kDa (Fluca) were used for calibration.

2.5. Scanning Electron Microscopy

The surfaces of the copolymer samples were studied using a scanning electron microscope JSM-IT 300 (Jeol Ltd., Tokyo, Japan) with an electron probe diameter of 5 nm (operating voltage 20 kV), using detectors of low-energy secondary electrons and backscattering electrons in low vacuum mode to prevent the samples from charging.

2.6. Elemental Analysis of Copolymers

The analysis of the samples was carried out by the CHNS analysis method on the Vario EL cube element analyzer for simultaneous determination of CHNS(O).

2.7. Freeze Drying

The sponges of the samples were obtained by lyophilic drying. The solvent was distilled under vacuum (1.33 Pa) using deep freezing by liquid nitrogen.

2.8. Tests of Polymer Films for Resistance to the Action of Microscopic Fungi

Tests for fungus resistance were carried out according to GOST [39], using method 1. Samples of the films were placed in a Petri dish; then, their surfaces were inoculated with a spore suspension of micromycete test cultures—active decomposers of polymeric

materials: Aspergillus oryzae F-2096, Aspergillus niger F-1119, Aspergillus terreus F-1025, Chaetomium globosum F-109, Paecilomyces variotii F-378, Penicillium funiculosum F-1115, Penicillium chrysogenum F-245, Penicillium cyclopium F-245, and Trichoderma viride A-1117. Petri dishes containing the prepared samples were placed in a thermostat. The tests were carried out for a period of 28 days at temperature of 29 ± 2 °C and humidity of more than 90%. At the end of the experimental period, the fungal resistance of the samples was evaluated on a 6-point scale (Table 2). The area of biofouling and the degree of micromycete development on the samples were taken into account for fungal resistance evaluations.

Table 2. Evaluation of the fungal resistance of samples.

Score	Score Characteristic
0	The germination of spores and conidia was not observed under a microscope
1	Germinated spores and slightly developed mycelium were visible under a microscope
2	A developed mycelium was clearly visible under a microscope; sporulation could be also observed
3	Mycelium and/or sporulation were barely visible to the naked eye, but clearly visible under a microscope
4	The development of fungi was clearly visible to the naked eye, covering less than 25% of the test surface
5	The development of fungi was clearly visible to the naked eye, covering more than 25% of the test surface

Samples are considered fungi-resistant if the growth of fungi does not exceed a score of 2.

3. Results and Discussion

It has previously been shown that grafting MMA on CC in an aqueous dispersion under visible light irradiation (λ = 400–700 nm) of the complex oxide $RbTe_{1.5}W_{0.5}O_6$ is associated with a series of transformations:

- The irradiated complex oxide generates electron–hole pairs, which leads to different radical formations [37,40]. There is a possibility of several chemical reactions occurring in the mixture. Collagen and MMA grafting are associated with an extremely active hydroxyl radical in the aqueous dispersion (Figure 1).
- The hydroxyl radical forms oxygen- or carbon-centered radicals, which are more stable than the HO• radical in the collagen macromolecule. It is related to separation of the hydrogen atom from the hydroxyl group in the amino acid residue (for example, hydroxyproline) or the hydrocarbon part of the amino acid residue molecule (for example, alanine) [41]. These radicals interact with the monomer and form a grafted synthetic fragment. Simultaneously, there is MMA grafting on the surface of $RbTe_{1.5}W_{0.5}O_6$, and the formation of the MMA polymer is due to the reaction between hydroxyl radical and monomer [10]. Schematically, the reactions that occurred in a mixture of catalyst, MMA, and CC are shown in Figure 1.

Evidence of PMMA grafting on collagen was the data of physicochemical analysis for the polymer isolated from the aqueous phase [10]. The curves of the molecular weight distribution (MWD) of the PMMA-CC graft copolymer were shifted to the region with high MW. Moreover, the MW values had increased in comparison to collagen. A comparison of the IR spectra for the PMMA-CC graft copolymer, collagen, and PMMA indicated that all of the characteristic bands of collagen and PMMA were observed for the graft copolymer. According to elemental analysis, the nitrogen content was decreased due to the grafting of a nitrogen-free synthetic fragment. The SEM results showed a new structural relief organization of the PMMA-CC graft copolymer in comparison to the initial collagen. This can be related to the compaction of the collagen fibers of the structural matrices.

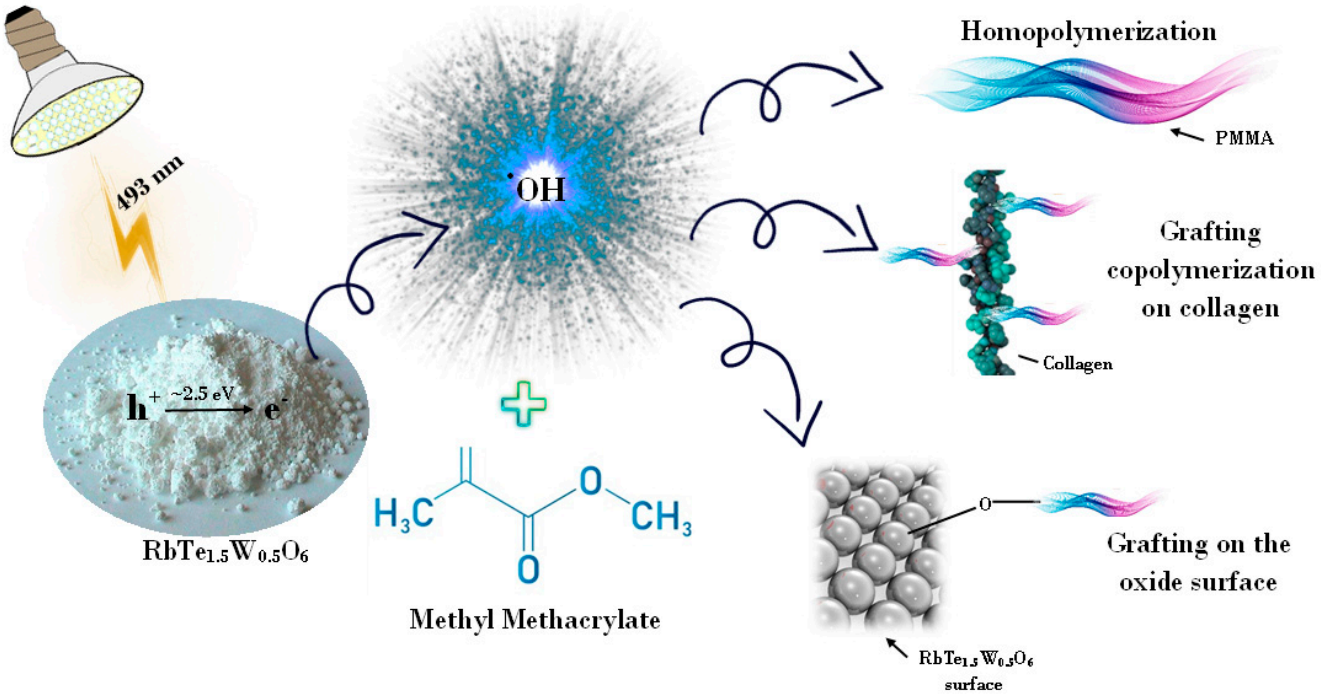


Figure 1. Schemes of different interactions between the hydroxyl radical and the substrate during photocatalytic polymerization.

The effect of adding modifiers to the structure and properties of collagen with acrylate graft copolymers was controlled by the same physicochemical characteristics. It is important to note that collagen isolated from any natural substrate, even the most delicately as a high-molecular-weight polymer with MW ~300 kDa, does not have sufficient mechanical strength. This is related to the destruction of intra- and intermolecular cross-links in a solution, which are formed during the biosynthesis of collagen in vivo and give its fibrils a stable structure, mechanical strength, and resistance to enzymes. In this regard, an important step in the creation of any collagen material is the development of cross-linking conditions that provide mechanical strength and stability for the duration that the material is used. In this case, cross-linking agents were used for collagen fibers of various natures [23–34].

This work considers collagen with copolymers of acrylates; therefore, a non-traditional approach was used. Cross-linking agents for acrylate fragments, such as TEDMA and AA, were added into the initial reaction mixture to form an additional ionic "crosslink" in the composite after neutralization of the final product. The formation of cross-links was expected (Figure 2a,b).

The synthesis of copolymer CCC-1 using photocatalysts was carried out similarly to that described earlier [10], by adding TEDMA and AA. The organic and aqueous phases were separated after synthesis. The aqueous phase consisted of a stable homogeneous solution of a white polymer product (Figure 3a). Purification of the grafted copolymer from AA and TEDMA residues was carried out by toluene extraction, and samples were dried in a vacuum. Insignificant amounts of organic polymer (less than 1%) were isolated from toluene extract after synthesis by precipitation with ethanol.

The study of the nitrogen content in the polymer product, isolated and neutralized from the aqueous phase, indicated the formation of collagen along with acrylate graft copolymer formation (Figure 4). This value, as well as that of the collagen content, were close to that of a copolymer without cross-linking additives. The results of the elemental analysis did not change after washing the copolymer with chloroform in the Soxlet extractor.

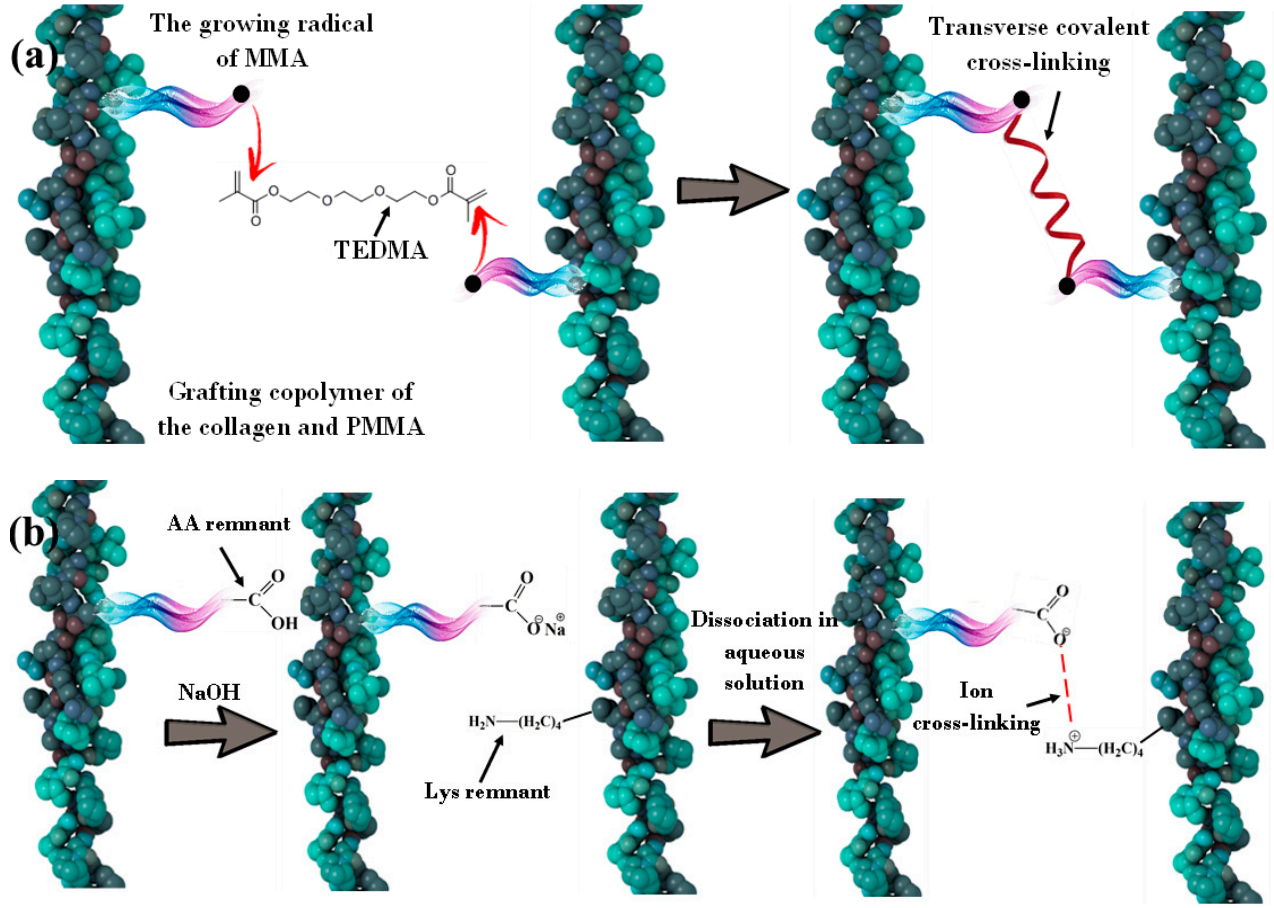
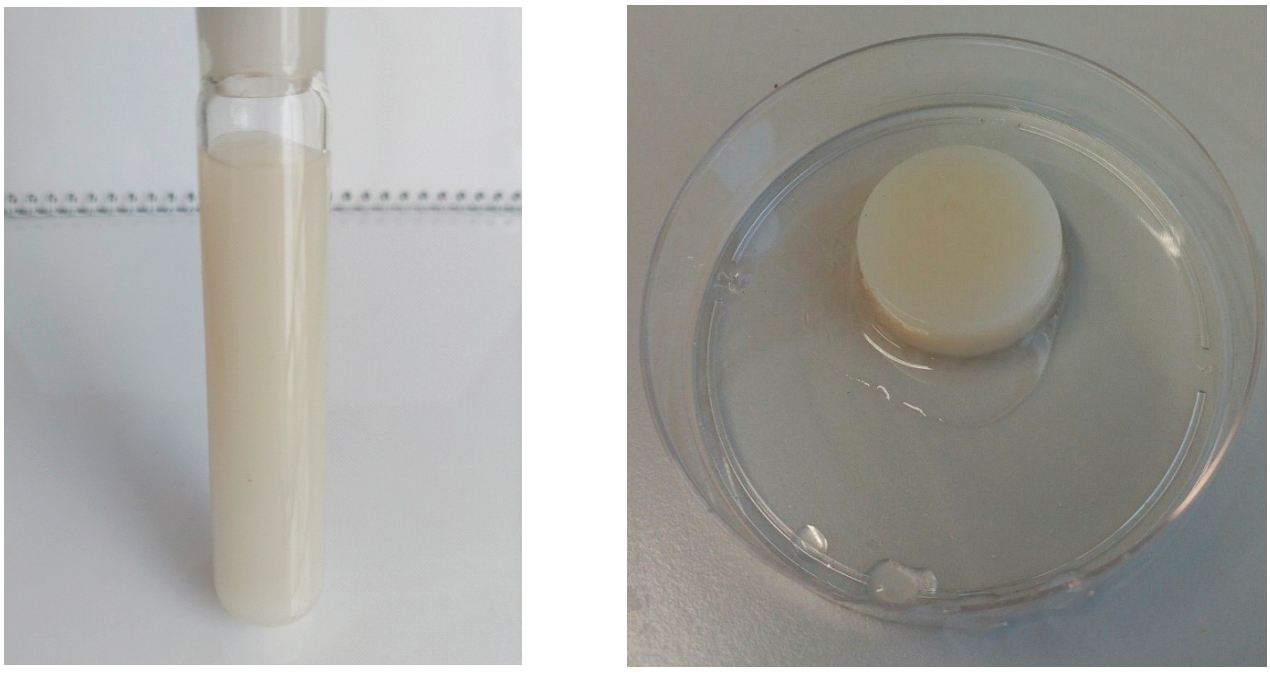


Figure 2. Scheme of (**a**) covalent cross-linking involving TEDMA; and (**b**) ionic cross-linking in a composite with acrylic acid fragments after neutralization of the final product.



(**a**) (**b**)

Figure 3. Photographs of (**a**) CCC-1 solution and (**b**) CCC-3 gel.

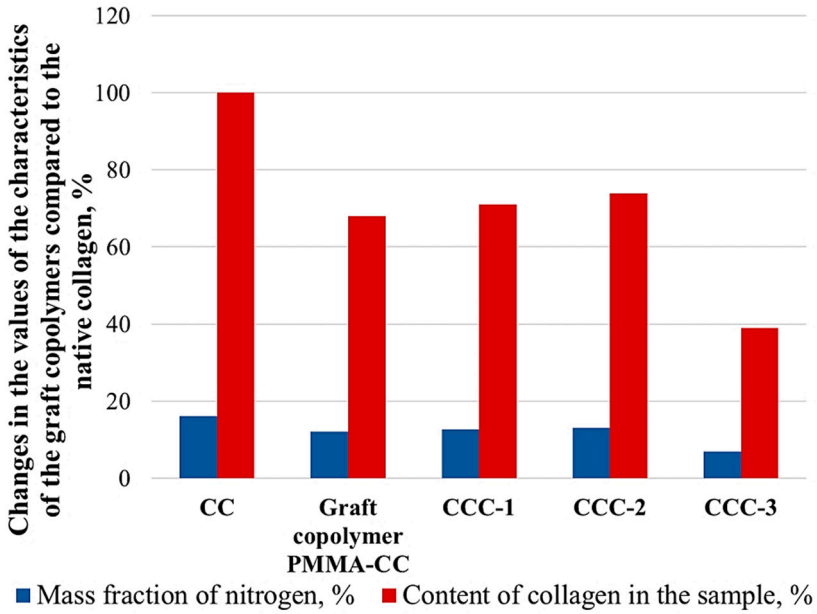


Figure 4. The contents of nitrogen and collagen in the samples, according to elemental analysis.

The most interesting information can be gathered from the SEM results of CCC-1 (Figure 5c,d) in comparison to the CC (Figure 5a) and PMMA-CC graft copolymers (Figure 5b). It is likely that, due to ionic cross-linking (Figure 2b) during the polymer solution neutralization and to the use of TEDMA, a matrix with a fine cellular structure was formed. Examples of such cross-linking have been described previously in the literature [24,26]. At high magnification, collagen fibers densified by the grafted synthetic fragment can be observed (Figure 5d).

The CCC-1 copolymer in water is a homogeneous phase, as shown in Figure 3a, and no coagulation occurs when an acidic solution is neutralized by alkali. At the same time, it was not possible to estimate the molecular weight parameters of the resulting CCC-1 copolymer, because it was retained on a filter with a pore size of 0.45 μm during the preparation of the polymer solution for GPC analysis. The filtered aqueous phase contained CCC-1 (~98% of the polymer product in solution), which was not integrated into the matrix, and low-molecular-weight collagen with MM ~10 kDa and ~20 kDa.

In the next series of experiments, the CC content was increased in order to suppress the formation of a synthetic polymer. In the case of the copolymer CCC-2, after synthesis, the organic phase was absent. This is extremely important because the process did not require an organic phase separation step. The aqueous phase, as in the previous experiment, consisted of a stable homogeneous solution of a white polymer product. The increase in CC in the reaction mixture led to a slight increase in the nitrogen content in the polymer in comparison to the first synthesis, as well as a corresponding decrease in the content of the synthetic fragment in the polymer. The organic polymer was not isolated from toluene extract after synthesis by precipitation with ethanol. A comparison of the SEM results for the CCC-2 samples (Figure 5f) with those previously described (Figure 5a–d) showed the formation of a matrix with a fine cellular structure. This can be explained by ionic cross-linking during the neutralization of the polymer solution with TEDMA, just as in the case of the CCC-1. It can be noted that the scatter of cell sizes was greater than that in the CCC-1 samples. Moreover, it was not possible to estimate the molecular weight parameters of the resulting CCC-2 copolymer because it remained on a filter with a pore size of 0.45 μm during the preparation of the polymer solution for GPC analysis. CCC-2 (~99% of the polymer product in solution) was observed in the filtered aqueous phase. It was not integrated into the matrix nor the low-molecular-weight collagen, with MM ~10 kDa and ~20 kDa.

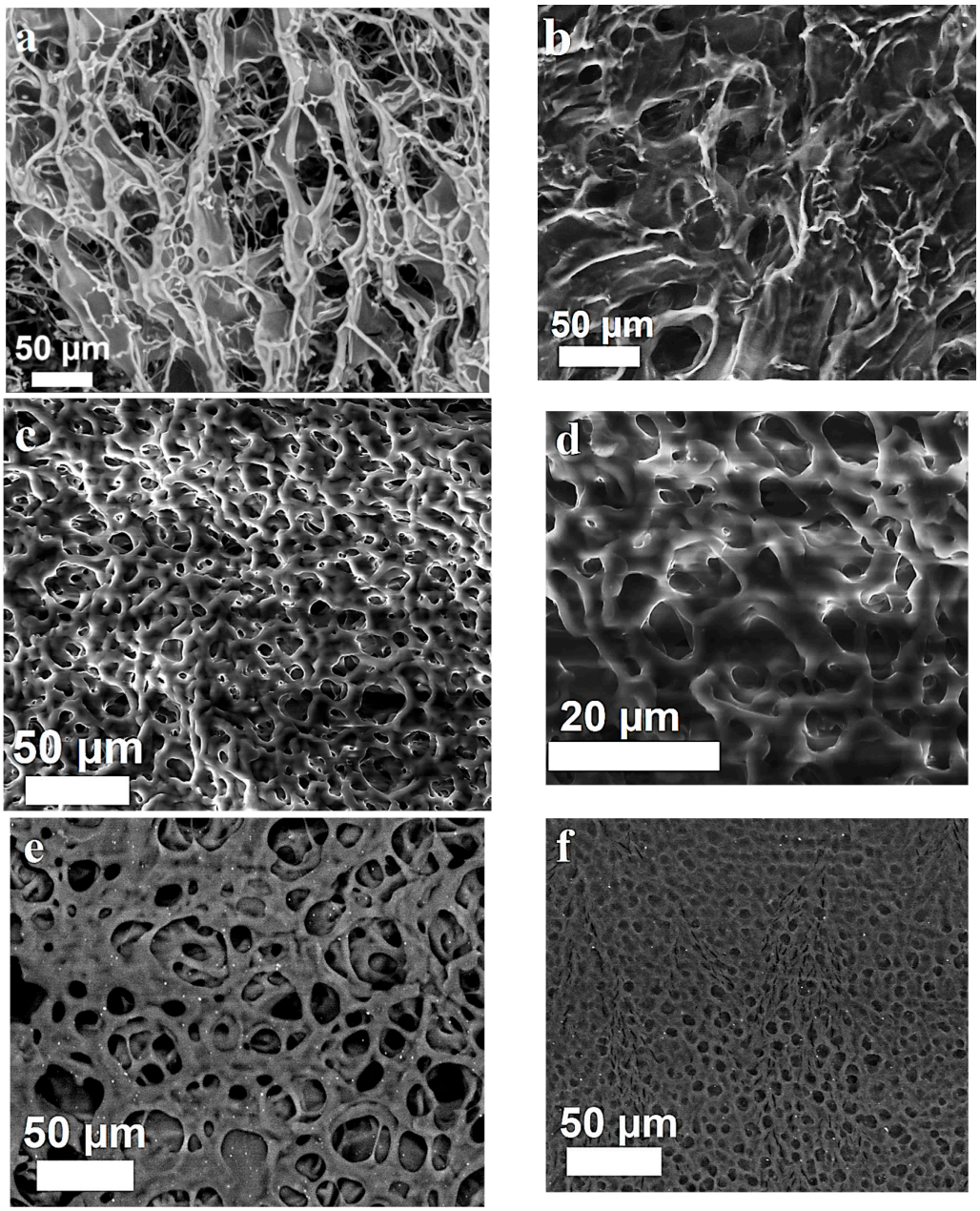


Figure 5. SEM images of (**a**) native collagen [10], (**b**) PMMA-CC graft copolymer [10], (**c**,**d**) CCC-1 graft copolymer, (**e**) CCC-2 graft copolymer, and (**f**) CCC-3 graft copolymer.

At the ratio of components in the CCC-3 copolymer, the organic phase was absent after the synthesis, just as in the case of CCC-2. Before adding the sodium hydroxide solution, the aqueous phase, as in the previous examples, consisted of a stable homogeneous solution of a white polymer product without delamination. However, after adding an alkali solution up to a pH of ~7, coagulation of the polymer product was observed with the gel formation (Figure 3b). The resulting gel easily released water; then, it was dried in a vacuum and formed a film made up of a polymer composite.

Gelation after PEG addition to the composition can be explained by additional radical processes. Firstly, it can occur due to interactions between PEG and hydroxyl radicals during synthesis, as in the case of collagen. Secondly, it can observed due to the detachment of a hydrogen atom from the hydroxyl group in PEG, according to scheme (1) (Figure 6). Finally, the hydrocarbon portion of the PEG molecule, according to scheme (2) (Figure 6), can form radicals in the PEG macromolecule.

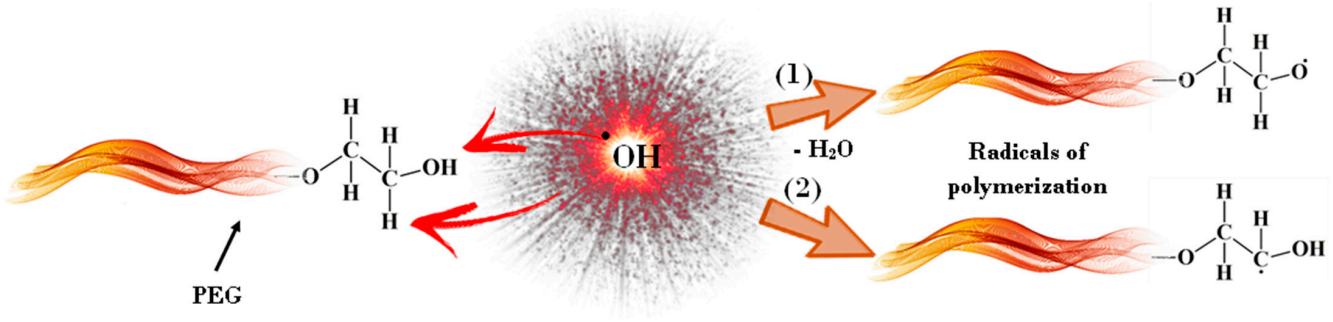


Figure 6. Formation of PEG radicals upon interaction with a hydroxyl radical.

These radicals were active in all radical transformations in the reaction mixture. They were able to interact with the monomer and form a grafted synthetic fragment, participate in the chain transfer reaction, or in the disproportionation reaction with another active radical, i.e., form additional covalent bonds in the resulting material. Moreover, as noted earlier [22], PEG formed covalent bonds with amino acid fragments of lysine and arginine (Figure 7).

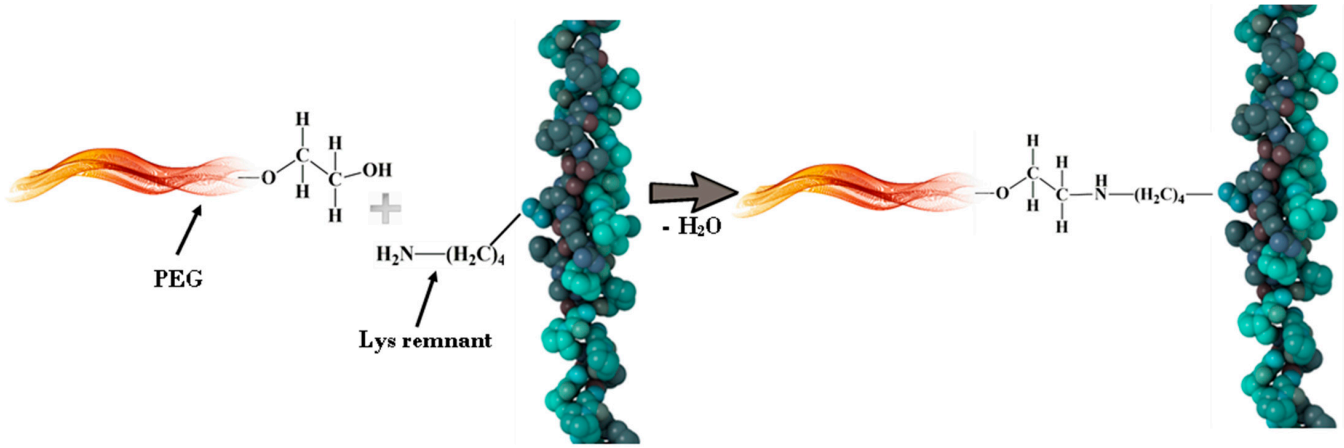


Figure 7. Scheme of the PEG interaction with amino acid residues of collagen, with the example of lysine.

In all cases, PEG acted as a neutral hydrophilic component to ensure steric stabilization of the polymer composite [17–22]. The chemical interactions during the preparation of new materials and the neutralization of the reaction mixture led to gel coagulation.

The addition of PEG into the reaction mixture led to a noticeable decrease in the nitrogen content and, accordingly, collagen in the polymer, in comparison to the previously obtained samples. SEM results for the CCC-3 (Figure 5f) samples showed a matrix with a fine cellular structure in comparison to CCC-1 and CCC-2 (Figure 5c–f). This was related to cross-linking chemical reactions in the reaction mixture occurring during the polymer solution neutralization. It can be noted that the morphology of CCC-3 was very similar to CCC-1 in terms of the cellular structure's uniformity.

Similarly to the previous cases with cross-linking agents in the synthesis, it was not possible to estimate the molecular weight parameters of the resulting CCC-3 copolymer, because it remained on a filter with a pore size of 0.45 μm while the polymer solution was prepared for GPC analysis. The filtered aqueous phase contained CCC-3 and PEG, which were not integrated into the matrix (with the ratio of graft copolymer to PEG being 1:1).

It is important to note that materials based on natural polymers, including those that contain collagen, are easily damaged by biological agents, such as microscopic fungi, under operating conditions [42,43]. It has already been determined that the PMMA-CC

copolymer has fungal resistance due to the content of $RbTe_{1.5}W_{0.5}O_6$ oxide particles in micro-amounts, with sizes of ≤100–200 nm [16]. It was necessary to determine whether the fungus-resistant properties of the material were preserved after adding specific components into the composite, such as PEG, AA, and TEDMA. The presence of dispersed catalyst particles can be seen in all SEM images in Figure 5. During the experiments, it was shown that the growth of fungi on polymer films CCC-1, CCC-2, and CCC-3 was 0 points. This suggests that these materials are not a food source for fungi.

Biostability is a critical property of medical polymer composites, because it determines the long-term function of a particular biomedical device.

4. Conclusions

The properties of collagen with graft copolymers of acrylates synthesized using photocatalysis with the complex oxide $RbTe_{1.5}W_{0.5}O_6$ and the addition of different known modifying additives were studied.

It was shown that the synthesis of cod collagen with acrylate graft copolymers, after adding triethylene glycol dimethacrylate in small volumes and acrylic acid in amounts comparable with the concentration of methyl methacrylate after neutralization, leads to the formation of a cross-linked polymer material that is readily soluble in water.

It has been established that the synthesis of cod collagen with acrylate graft copolymers after adding triethylene glycol dimethacrylate in small volumes, acrylic acid in an amount comparable with the concentration of methyl methacrylate, and polyethylene glycol in an amount comparable with the amount of collagen after neutralization, leads to a coagulate with a cross-linked structure. The gel easily releases water while being dried in a vacuum, and forms a film of polymer composite.

Composite materials based on fish collagen with modifiers such as TEDMA, AA, and PEG, obtained by photocatalysis, are fungus-resistant.

Thus, the results indicate the feasibility of using modified materials based on copolymers of methyl methacrylate and fish collagen, prepared by photocatalysis in the presence of $RbTe_{1.5}W_{0.5}O_6$ oxide, to obtain a wide range of medical products, including colloidal solutions, gels, and films. The fungal resistance of materials during product operation is especially important.

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Data Availability Statement: The data presented in this study are available upon request from the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Gold, M.H.; Sadick, N.S. Optimizing outcomes with polymethylmethacrylate fillers. *Cosmet. Dermatol.* **2018**, *17*, 298–304. [CrossRef]
2. Nishimura, S.; Hokazono, N.; Taki, Y.; Motoda, H.; Morita, Y.; Yamamoto, K.; Higashi, N.; Koga, T. Photocleavable peptide−poly(2-hydroxyethyl methacrylate) hybrid graft copolymer via postpolymerization modification by click chemistry to modulate the cell affinities of 2D and 3D materials. *ACS Appl. Mater. Interfaces* **2019**, *11*, 24577–24587. [CrossRef] [PubMed]

3. Yu, F.; Cheng, S.; Lei, J.; Hang, Y.; Liu, Q.; Wang, H.; Yuan, L. Heparin mimics and fibroblast growth factor-2 fabricated nanogold composite in promoting neural differentiation of mouse embryonic stem cells. *J. Biomater. Sci. Polym. Ed.* **2020**, *31*, 1623–1647. [CrossRef]
4. Qi, D.; Wu, S.; Kuss, M.A.; Shi, W.; Chung, S.; Deegan, P.T.; Kamenskiy, A.; He, Y.; Duan, B. Mechanically robust cryogels with injectability and bioprinting supportability for adipose tissue engineering. *Acta Biomater.* **2018**, *74*, 131–142. [CrossRef]
5. Gamo, R.; Pinedo, F.; Viecente, J.; Naz, E.; Calzado, L.; Ruiz-Genao, D.; Gomez de la Fuente, E.; Alvarez, G.; Arranz, E.; Lopez-Estebaranz, J.-L. Keratoacanthoma-like reaction after a hyaluronic acid and acrylic hydrogel cosmetic filler. *Dermatol. Surg.* **2008**, *34*, 954–959. [CrossRef]
6. Wolfram, D.; Tzankov, A.; Piza-Katzer, H. Surgery for foreign body reactions due to injectable fillers. *Dermatology* **2006**, *213*, 300–304. [CrossRef]
7. Mukhametov, U.F.; Lyulin, S.V.; Borzunov, D.Y.; Gareev, I.F.; Beylerli, O.A.; Yang, G. Alloplastic and implant materials for bone grafting: A literature review. *Creat. Surg. Oncol.* **2021**, *11*, 343–353. [CrossRef]
8. Nashchekina, Y.A.; Lukonina, O.A.; Mikhailova, N.A. Chemical crosslinking agents for collagen: Interaction mechanisms and prospects for use in regenerative medicine. *Cytology* **2020**, *62*, 459–472. [CrossRef]
9. Kadimaliev, D.; Parchaykina, O.; Zamylina, L.; Cousin, E.; Mamin, B.; Mishkin, V.; Marisova, Y. Biodegradable Film. RF Patent No. 2564824 C1, 10 October 2015.
10. Semenycheva, L.; Chasova, V.; Fukina, D.; Koryagin, A.; Valetova, N.; Suleymanov, E. Synthesis of polymethyl-methacrylate–collagen-graft copolymer using a complex oxide $RbTe_{1.5}W_{0.5}O_6$ photocatalyst. *Polym. Sci. Ser. D* **2022**, *5*, 110–117. [CrossRef]
11. Semenycheva, L.; Uromicheva, M.; Chasova, V.; Fukina, D.; Koryagin, A.; Valetova, N.; Suleymanov, E. Synthesis of grafted polybutylacrylate copolymer on fish collagen using a photocatalyst—Complex oxide RbTe1,5W0,5O6. *Proc. Univ. Appl. Chem. Biotechnol.* **2022**, *12*, 97–108. [CrossRef]
12. Uromicheva, M.A.; Kuznetsova, Y.L.; Valetova, N.B.; Mitin, A.V.; Semenycheva, L.L.; Smirnova, O.V. Synthesis of grafted polybutyl acrylate copolymer on fish collagen. *Proc. Univ. Appl. Chem. Biotechnol.* **2021**, *11*, 16–25. [CrossRef]
13. Chasova, V.; Semenycheva, L.; Egorikhina, M.; Charykova, I.; Linkova, D.; Rubtsova, Y.; Fukina, D.; Koryagin, A.; Valetova, N.; Suleymanov, E. Gelatin as an alternative to cod collagen in hybrid materials for regenerative medicine. *Macromol. Res.* **2022**, *30*, 212–221. [CrossRef]
14. Semenycheva, L.; Chasova, V.; Fukina, D.; Koryagin, A.; Belousov, A.; Valetova, N.; Suleimanov, E. Photocatalytic synthesis of materials for regenerative medicine using complex oxides with β-pyrochlore structure. *Life.* **2023**, *13*, 352. [CrossRef]
15. Chasova, V.; Fukina, D.; Buryakov, A.; Zhizhin, E.; Koroleva, A.; Semenycheva, L.; Suleymanov, E. The effect of transformations of methyl methacrylate during photocatalysis in the presence of $RbTe_{1,5}W_{0,5}O_6$ on the change in the surface of the complex oxide. *Proc. Univ. Appl. Chem. Biotechnol.* **2022**, *12*, 208–221. [CrossRef]
16. Suleymanov, E.; Chasova, V.; Valetova, N.; Semenycheva, L.; Fukina, D.; Koryagin, A.; Smirnov, V.; Smirnova, O. Method of Obtaining Methylmethacrylate Copolymers. RF Patent No. 2777896 C1, 11 August 2022.
17. Nikitin, I.G.; Baykova, I.E.; Gogova, L.M. Pegylated medicinal preparations: The current state of the problem and prospects. *Lechebnoye Delo.* **2005**, *4*, 18–24.
18. Porfirieva, N.N.; Mustafina, R.I.; Khutoryansky, V.V. Pegylated systems in pharmaceuticals. *Polym. Sci. Ser. C* **2020**, *62*, 66–80. [CrossRef]
19. Martinez, A.P.; Qamar, B.; Marin, A.; Fuerst, T.R.; Muro, S.; Andrianov, A.K. Biodegradable "Scaffold" polyphosphazenes for non-covalent PEGylation of proteins. In *Polyphosphazenes in Biomedicine, Engineering, and Pioneering Synthesis*; Andrianov, A., Allcock, H., Eds.; ACS Symposium Series; American Chemical Society: Washington, DC, USA, 2018; pp. 121–141. [CrossRef]
20. Muthulakshmi, L.; Rajulu, A.V.; Ramakrishn, S.; Thomas, S.; Pruncu, C.I. Development of biocomposite films from natural protein sources for food packaging applications: Structural characterization and physicochemical properties. *J. Appl. Polym. Sci.* **2022**, *139*, 51665. [CrossRef]
21. Niu, Y.; Liu, G.; Fu, M.; Chen, C.; Fu, W.; Zhang, Z.; Xia, H.; Stadler, F.J. Designing a multifaceted bio-interface nanofiber tissue-engineered tubular scaffold graft to promote neo-vascularization for urethral regeneration. *J. Mater. Chem. B* **2020**, *8*, 1748–1758. [CrossRef]
22. Zalipsky, S. Active Carbonates of Polyalkylene Oxides for Modification of Polypeptides. US Patent No. 5122614A, 16 June 1992.
23. Gelli, R.; Mugnaini, G.; Bolognesi, T.; Bonini, M. Cross-linked porous gelatin microparticles with tunable shape, size, and porosity. *Langmuir* **2021**, *37*, 12781–12789. [CrossRef] [PubMed]
24. Cao, J.; Wang, Y.; He, C.; Kang, Y.; Zhou, J. Ionically crosslinked chitosan/poly(acrylic acid) hydrogels with high strength, toughness and antifreezing capability. *Carbohyd. Polym.* **2020**, *242*, 116420. [CrossRef]
25. Fatkhutdinova, N.L.; Vasiliev, A.V.; Bukharova, T.B.; Osidak, E.O.; Starikova, N.V.; Domogatsky, S.P.; Kulakov, D.V.; Goldstein, A.A. Prospects of using collagen hydrogel as a base for cured and activated bone-plastic materials. *Stomatologiia* **2018**, *6*, 78–83. [CrossRef]
26. Park, K.M.; Park, K.D.; Sevastianov, V.I.; Nemetz, E.A.; Vasilets, V.N. In situ crosslinkable hydrogels for engineered cellular microenvironment. *Regen. Med. Cell. Technol.* **2017**, *19*, 53–64. [CrossRef]
27. Wang, G.; Lin, Z.; Jin, S.; Li, M.; Jing, L. Gelatin-derived honeycomb like porous carbon for high mass loading supercapacitors. *J. Energy Storage.* **2022**, *45*, 103525. [CrossRef]

28. Guan, X.; Zhang, B.; Li, D.; He, M.; Han, Q.; Chang, J. Remediation and resource utilization of chromium(III)-containing tannery effluent based on chitosan-sodium alginate hydrogel. *Carbohydr. Polym.* **2022**, *284*, 119179. [CrossRef]
29. Dhingra, K.; Dinda, A.K.; Kottarath, S.K.; Chaudhari, P.K.; Verma, F. Mucoadhesive silver nanoparticle-based local drug delivery system for peri-implantitis management in COVID-19 era. Part 1: Antimicrobial and safety in-vitro analysis. *J. Oral Biol. Craniofac. Res.* **2022**, *12*, 177–181. [CrossRef] [PubMed]
30. Rana, T.; Fatima, M.; Khan, A.Q.; Naeem, Z.; Javaid, S.; Sajid, N.; Habib, A. Hydrogels: A novel drug delivery system. *J. Biomed. Res. Environ. Sci.* **2020**, *1*, 439–451. [CrossRef]
31. Saha, R.; Patkar, S.; Maniar, D.; Pillai, M.M.; Tayalia, P. A bilayered skin substitute developed using an eggshell membrane crosslinked gelatin–chitosan cryogel. *Biomater. Sci.* **2021**, *9*, 7921–7933. [CrossRef] [PubMed]
32. Do Livramento Linhares Rodrigues Menezesa, M.; da Rocha Pires, N.; Rodrigues da Cunha, P.L.; de Freitas Rosa, M.; Silva de Souza, B.W.; Pessoa de Andrade Feitosa, J.; de Sá Moreira de Souza Filho, M. Effect of tannic acid as crosslinking agent on fish skin gelatin-silver nanocomposite film. *Food Packag. Shelf Life.* **2019**, *19*, 7–15. [CrossRef]
33. Liguori, A.; Uranga, J.; Panzavolta, S.; Guerrero, P.; de la Caba, K.; Focarete, M.L. Electrospinning of fish gelatin solution containing citric acid: An environmentally friendly approach to prepare crosslinked gelatin fibers. *Materials* **2019**, *12*, 2808. [CrossRef]
34. Moghadas, B.; Solouk, A.; Sadeghi, D. Development of chitosan membrane using non-toxic crosslinkers for potential wound dressing applications. *Polym. Bull.* **2021**, *78*, 4919–4929. [CrossRef]
35. da Silva, M.T.S.; Pinto, J.C. Production of crosslinked gelatin beads in inverse suspension processes. *Polym. Eng. Sci.* **2018**, *59*, 519–525. [CrossRef]
36. Medum. Available online: https://medum.ru/e1521 (accessed on 16 March 2023).
37. Fukina, D.G.; Koryagin, A.V.; Koroleva, A.V.; Zhizhin, E.V.; Suleimanov, E.V.; Kirillova, N.I. Photocatalytic properties of β-pyrochlore $RbTe_{1.5}W_{0.5}O_6$ under visible-light irradiation. *J. Solid State Chem.* **2021**, *300*, 122235. [CrossRef]
38. Semenycheva, L.L.; Kuznetsova, J.L.; Valetova, N.B.; Geras'kina, E.V.; Tarankova, O.A. Method for Production of Acetic Dispersion of High Molecular Fish Collagen. RF Patent No. 2567171 C1, 10 November 2015.
39. Kirillov, V.N.; Berenson, V.F.; Krashakov, Y.F.; Skribachilin, V.B.; Semenov, S.A.; Popovkin, B.A.; Ugarova, N.N.; Brovko, L.Y.; Polyakova, A.V.; Ivanova, I.G.; et al. Unified system of corrosion and ageing protection. Polymer materials and their components. Methods of laboratory tests for mould resistance. Gosstandart of the USSR, no. 9.049-91, 1991. Available online: https://allgosts.ru/19/040/gost_9.049-91 (accessed on 1 March 2023).
40. Fukina, D.G.; Suleimanov, E.V.; Boryakov, A.V.; Zubkov, S.Y.; Koryagin, A.V.; Volkova, N.S.; Gorshkov, A.P. Structure analysis and electronic properties of $ATe^{4+}_{0.5}Te^{6+}_{1.5-x}M^{6+}_{x}O_6$ (A=Rb, Cs, M^{6+}=Mo,W) solid solutions with β-pyrochlore structure. *J. Solid State Chem.* **2021**, *293*, 121787. [CrossRef]
41. Semenycheva, L.L.; Egorikhina, M.N.; Chasova, V.O.; Valetova, N.B.; Fukina, D.G.; Sukhareva, A.A.; Ostrosablin, A.N.; Kobyakova, I.I.; Farafontova, E.A.; Rubtsova, Y.P. An environmentally friendly solution related to the use of fish production waste to manufacture new materials for biomedicine. *Examines Mar. Biol. Oceanogr.* **2022**, *5*, 000606. [CrossRef]
42. Erofeev, V.T.; Smirnov, V.F.; Morozov, E.A. *Microbiological Degradation of Materials*; Association of Construction Universities: Moscow, Russia, 2008; p. 123.
43. Eliazyan, G.A.; Markaryan, S.M.; Paronikyan, A.E.; Margaryan, L.Y. Study of biocidal treatment of the leather of medieval book covers by the method of retanning with an aluminum complex stabilized with trimethylolmelem. *Proc. NPU Armen. Metall. Mater. Sci. Min. Eng.* **2021**, *1*, 1–82.

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