Preparation and characterization of keratin-based biocomposite hydrogels prepared by electron beam irradiation

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ABSTRACT

The biocompatible and highly porous keratin-based hydrogels were prepared using electron beam irradiation (EBI). The conditions for keratin-based hydrogel formation were investigated depending on several conditions, including the presence of poly(vinyl alcohol) (PVA), concentration of keratin solution, EBI dose, and poly(ethylene imine) (PEI) additives. The pure keratin (human hair and wool) aqueous solution was not gelled by EBI while the aqueous keratin solutions blended with PVA were gelled at an EBI dose of more than 90 kGy. Furthermore, in the presence of PEI, the aqueous keratin solution blended with PVA could be gelled at a considerably lower EBI dose, even at 10 kGy. This finding suggests that the PEI additives significantly influence the rate of gelation and that PEI functions as an accelerator during gelation. The resulting keratin-based hydrogels were characterized using scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FT-IR), gel fraction, degree of swelling, gel strength, and kinetics of swelling analyses.

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1. Introduction

Throughout history, humans have actively exploited natural resources that were once deemed inexhaustible. Fossil fuels have become the driving force for civilization since the industrial revolution and ongoing rapid global urbanization. Following the industrial revolution, humans have invented numerous useful technologies that have continued to increase their average life expectancy. This increase in life expectancy has given rise to considerable environmental concerns and issues that we should address for the sake of posterity. Recently, we have attempted to protect the earth and investigate environmental problems and solutions. One environmentally-friendly material is keratin. Keratin proteins are structural fibrous proteins that are the key structural component of hair, feathers, wool, nails, and horns [1]. These proteins have three-dimensional mesh structures. Keratin extracted from waste hair or wool has been investigated as a suitable material for use in biological organisms [2,3]. Keratins are composed of cysteine-rich structural proteins, which have a considerable number of disulfide bonds. Furthermore, keratin has significant mechanical properties because of its hard fibrous structure. There are more than 300,000 tons of unused protein-rich hair wastes produced worldwide each year. These waste keratins are available for creating water-soluble compounds through the reduction of disulfide bonds [4,5]. A significant amount of data has been collected on the function and composition of materials produced using keratins [6–8].

Hydrogels are three-dimensional polymer networks in which hydrophilic polymer chains are connected by physical or chemical bonds. These bonds underlay the integrity and physical stability of the networks, whereas the thermodynamic compatibility of the polymer chains with water allows these materials to swell in aqueous solvents. There are numerous applications for hydrogels [9–13], particularly in the medical and pharmaceutical sectors. Because of their biocompatibility and high water content, hydrogels, which are a soft and wet material, can be used in such applications as tissue engineering, drug delivery devices, contact lenses, and materials for artificial skin. The majority of hydrogels are produced using chemical or physical methods [14–16]. In chemical processes, hydrogels are produced using chemical reagents, catalysts, temperature, and time to achieve a desirable degree of cross-linking. However, the chemicals used to produce hydrogels are generally toxic; therefore, post-processing treatments are required to remove these toxic chemicals, leading to environmental contamination caused by wastewater from the post-processing procedures. In contrast, physical processes result in less environmental contamination, use less energy and waste less water. Along these lines, radiation techniques are useful for preparing hydrogels, and this technique simultaneously combines cross-linking and sterilization effects [17–19]. UV-radiation and ionizing radiations, such as gamma radiation and electron beams (EB), have been widely used to accomplish cross-linking. The use of cross-linkers and toxic reagents are not necessary when hydrogels are produced using radiation; therefore, radiation-treated hydrogels can
be used in wound dressings without further post-processing[17–19]. To date, various polymers have been used to produce hydrogels [20,21]. These include not only synthetic polymers such as poly(acrylic acid) (PAA), poly(acrylamide) (PAAm) [22], poly(hydroxyethyl methacrylate), poly(ethylene glycol) (PEG), poly(vinyl pyrrolidone) (PVP), poly(vinyl alcohol) (PVA), and poly(ethylene oxide) (PEO) but also natural polymers (agar, alginate, collagen, and chitosan). In particular, poly(vinyl alcohol) (PVA), which is a nonionic, hydrophilic, nontoxic, water-soluble, and biocompatible polymer, has been widely used as a colloid stabilizer; as a coating in the textile industry; and as a material in the production of various hydrogels, membranes, and industrial and medical devices [23]. PVA hydrogels are suitable for industrial applications because of their adaptability for large-scale manufacturing processes and their considerable mechanical properties [21]. Although there are numerous reports on PVA-blended materials [24–28], to date, there are a few reports on the hydrogels based on human hair and wool [29–32]. A keratin hydrogel can be used as a wound dressing and cell scaffolding because they are abundant and bioactive and are a realistic source of autologous proteins [33]. In this study, we attempted to create biocompatible hydrogels using natural polymer wastes, such as human hair and wool, for the first time by EBI. We report the preparation of keratin protein hydrogels based on human hair and wool blended with PVA by EBI and the corresponding mechanical properties, gel fraction, and swelling behavior. The objectives of this study were to reduce environmental contaminations and to produce environmentally friendly hydrogels.

2. Experimental

2.1. Materials

Urea, sodium dithionite (Na2S2O5), sodium dodecyl sulfate (SDS), acetonitrile (CH3CN), ethanol (C2H5OH), poly(ethylene imine) (PEI), branched, average Mw ~ 10,000 g/mol by GPC, average Mn ~ 25,000 g/mol by light scattering) and poly(vinyl alcohol) (PVA) (Mw ~ 85,000–124,000 g/mol, 87–89% hydrolyzed) were purchased from the Sigma Aldrich, Co. (St. Louis, USA). Human hair was obtained from local hair salons for free, and waste wool (Merino) was provided by local bedding factories. Double-distilled water was used for creating aqueous solutions. Disposable sterilized polystyrene square dishes were purchased from the SPL Life Sciences Co., Republic of Korea. The size of dishes was 125 × 125 × 20 mm.

2.2. Keratin extraction method (S-sulfo keratin)

Wool (Merino) and human hair were thoroughly washed with water that contained 0.5% SDS, rinsed with fresh water and then air-dried. Keratin was extracted by sulfitolysis [34,35]. To remove external lipids and impurities, the cleaned hair and wool were separately extracted using a Soxhlet apparatus with ethanol and acetone for 12 h. The dried hair and wool samples (150 g each) were cut into snippets of some millimeters and then placed into 1.5 L of an aqueous solution that contained 8 M urea, 75 g of SDS and 150 g of Na2S2O5. The mixture was heated to 100 °C, shaken for 30 min and then cooled in a water bath at 30 °C. The resulting mixture was filtered through a stainless-steel mesh with a pore size of 75 μm. The filtrate was dialyzed against 15 L of water that contained 0.1 wt.% Na2S2O5 using cellulose tubing (molecular-weight cutoff of 12,000 Da) for 3 days. The outer solution was changed twice per day. The protein concentration in the dialyzed solution was determined using the bicinchoninic acid (BCA) assay method with a QuantiPro™ BCA Assay Kit (Sigma-Aldrich, St. Louis, USA). The concentration of keratin was measured using the Bradford protein assay (Bio-Rad) with bovine serum albumin as a standard [36]. All measurements were performed at 25 °C. Fig. 1 presents the protein standard curve (correlation coefficient of r ~ 0.9988), which was obtained by plotting the absorbance versus the amount of protein standards.

Duplicate or triplicate sample readings were averaged, and the protein concentrations in the human hair and wool were extrapolated from the standard curve. Here, the standard deviation of the estimated protein concentrations should be ≤5%. The protein concentration in the dialyzed (10 wt.% hair or wool in aqueous solution) was 5.0 wt.% on average using the BCA quantitation method. The UV absorption spectra of the S-sulfo keratin extracted from the hair and wool samples were investigated (inset in Fig. 1). We observed that the characteristic protein peak was clearly visible at 562 nm due to the formation of a complex between copper ions and peptide bonds to produce a purple end product [36–38]. This result suggested that the S-sulfo keratin was not denatured during sulfitolysis and post-treatment processing.

2.3. Preparation of keratin hydrogels

Hydrogels that contained the keratin protein were produced in the form of a sheet (125 mm × 125 mm × 5 mm, width × length × thickness). Briefly, in order to improve the gelation, the PVA solution was blended with S-sulfo keratin solution with various weight fractions of S-sulfo keratin. Furthermore, 0.01 wt.% PEI was included in the PVA solution. Afterwards, the S-sulfo keratin/PVA blended solution was poured into the square dishes, and the dishes were irradiated with an electron-beam at a dose of 10 kGy–100 kGy. The irradiation of the samples was performed using an electron beam accelerator (beam energy of 2.5 MeV, beam current of 8.5 mA, irradiation width of 110 cm, conveyor velocity of 10 m/min, dose rate of 6.67 kGy/s, roller type handling system, EBTECH Co., Ltd., Korea) at room temperature in an air atmosphere. After gelation, the hydrogels were cut into the required sizes for conducting various analyses. With respect to the radiation dose unit, 1 kilogram (kGy) equals the absorption of 1 kilojoule (kJ) or 1 kilowatt second (kWs) per kilogram (kg) of material. The specific energy (SE) requirement in kilojoules per kilogram (kJ/kg) is simply equal to the dose in kilogram, as follows [37]:

\[
SE = D \text{[kJ/kg].}
\]  

2.4. Gel strength

The tensile strength at rupture and the elongation at break were measured using a Universal Test Machine (UTM) (Lloyd, US/LRIOK) according to the ASTM D882 standard method. Strips with dimensions of 50 mm × 15 mm × 5 mm were prepared by cutting a portion from the hydrogel. A cross-head speed of 50 mm/min was used. The data
were transferred to a computer for evaluating the stress–strain curve. The gel strength was calculated using Eq. (2) [39].

\[
\text{Gel strength (g cm⁻¹)} = \frac{\text{tensile strength at rupture}}{\text{elongation at break}}
\]  

(2)

2.5. Degree of swelling

After gelation, the samples of the hydrogel were removed from the trays, dried, weighed \((w_1)\) and then placed into distilled water until the sample reached the equilibrium degree of swelling. After 72 h, the samples were removed from the water. The excess water was wiped from the surface with blotting paper, and the swollen samples were weighed \((w_2)\). Afterwards the samples were dried and weighed \((w_3)\). The % water uptake was calculated using Eq. (3).

\[
\% \text{ degree of swelling} = \left(\frac{w_2 - w_3}{w_1}\right) \times 100
\]

(3)

2.6. Gel fraction

After gelation, the cross-linked hydrogels were dried at 60 °C for 48 h until the hydrogel reached a constant weight \((A)\). The hydrogels were boiled in double-distilled water for 5 min. The insoluble part of the hydrogel was washed again in distilled water and then dried to a constant weight \((B)\) [40]. The % gel fraction was then calculated using Eq. (4).

\[
\% \text{ gel fraction} = \left(\frac{B}{A}\right) \times 100
\]

(4)

2.7. Characterization

The changes in the functional groups of the hydrogels that may have been caused by the physical or chemical treatments were evaluated using Fourier transform infrared spectroscopy (FT-IR, Varian 1000 FT-IR Scimitar series, PIKE Technologies, USA) on freeze dried samples. A standard procedure was used to create hydrogel samples for scanning electron microscopy (SEM) measurements. Freeze-dried hydrogel samples were coated with platinum (ion-sputter, Hitachi E-1010, Japan) under vacuum and were used to investigate the morphology of keratin protein hydrogels. SEM images of the surface and fractured morphologies of the hydrogels were recorded at 15 kV with different magnifications using a Jeol JSM-5900 SEM machine.

3. Results and discussion

3.1. Keratin-based hydrogels

In general, radiation induces scission reactions of the polymer chains and/or cross-linking reactions by mutually recombining the generated free-radicals [17–21]. Both the intermolecular and intramolecular covalent bonds formed in polymer networks are primarily dependent on the concentration of the polymer, the types of polymers involved, and the applied irradiation dose. In our system, we successfully prepared human hair- and wool-based hydrogels using EBI. The formation of such keratin-based hydrogels was significantly dependent on several conditions, including the presence of PVA, concentration of the keratin solution, EBI dose, and PEI additives. When S-sulfo keratin is extracted from human hair and wool through sulfotolysis with sodium disulfite, the cystine disulfide bonds are cleaved by the sulfite to form cysteine thiol (reduced keratin) [33,34]. Afterwards, the aqueous S-sulfo keratin solutions were blended with PVA to form the hydrogels through covalent cross-linking on irradiation [34]. Interestingly, in this work, the pure keratin (human hair and wool) aqueous solutions were not gelled while the aqueous keratin solution blended with PVA was only gelled above 90 kGy, but not below 90 kGy. In contrast, the aqueous keratin solution blended with PVA in the presence of PEI gelled at a considerably lower EBI dose (even at 10 kGy), an amount that is normally insufficient for inducing gelation by irradiation [17–21]. Although not fully understood, this result suggests that the PEI additives significantly influenced the rate of gelation and that PEI served as an accelerator during gelation. Compared to the corresponding keratin hydrogel without the addition of PEI, the addition of a small amount of 0.01 wt.% PEI exerted a considerable effect on decreasing the EBI dose for the formation of hydrogels. This finding may be attributed to physical cross-linking [41] among S-sulfo keratin main chains, oxygen groups in PVA aqueous solution and the amine groups of PEI, and then covalent cross-linking [41] between the corresponding protein chains under electron beam irradiation. However, above 0.01% PEI, S-sulfo keratin was severely aggregated and failed to form a hydrogel. Generally, polyethylenimine (PEI), which is cationic polyelectrolyte, has been utilized in many applications, including adhesives, films, polymer modification, wastewater treatment [40–48] and making the plasmids leading to high transfection efficiency [49,50]. In our system, it was expected that PEI could bind ionically to the protein [51–53], and this would accelerate gel formation and would be also consistent with the increase in aggregation of the proteins with increasing the concentration of PEI. Optimal concentrations of the S-sulfo keratin and PVA solutions were 5.0 wt.% and 5.0 wt.% in the total weight of the solution, respectively.

3.2. Morphologies

Fig. 2 presents SEM micrographs of freeze-dried hydrogels produced from human hair and wool solutions blended with PVA in the presence of PEI at a dose of 10 kGy. As seen in Fig. 2, freeze-dried human hair-based and wool-based hydrogels clearly exhibited porous structures compared to the pure PVA hydrogels. Furthermore, the human hair-based hydrogel exhibited a considerably larger pore size than that of the wool-based hydrogels. Pore size can influence the physico-chemical properties of the resulting hydrogels, such as the gel fraction, degree of swelling, gel strength, and swelling kinetics. However, the PVA hydrogels exhibited uniform surface morphologies, indicating the presence of a homogeneous hydrogel network. SEM images demonstrated that the types of protein and its contents strongly influenced the morphological properties of the resulting hydrogels [54].

3.3. FT-IR spectra

Fig. 3 presents the FT-IR spectra of human hair-based and wool-based hydrogels and pure PVA hydrogels prepared using EBI in the presence of PEI. In the FT-IR spectra of the intact keratin proteins (human hair and wool before irradiation), typical characteristic peaks of the keratin proteins were observed at 1625 cm⁻¹, which corresponds to the elastic vibration of the C=O bond, and at 1520 cm⁻¹, which corresponds to the bending deformation of the C–N – H bond [55]. We can observe the O–H flexing liberation peak at 3400 cm⁻¹ and the N–H stretching vibrations of the amine group at 3300 cm⁻¹. Furthermore, while hair-based hydrogels exhibited absorption peaks similar to those of keratin proteins, wool-based hydrogels exhibited a slight shift for the typical absorption peaks of the C=O and C–N – H bonds. This result indicated that stronger interactions between the polymer backbones in the wool and the amine groups in PEI or the –OH groups in PVA were formed; however, the same was not true for human hair. These results were in good agreement with results from the SEM observations, which indicated that hair-based hydrogels presented coarser morphologies than did wool-based hydrogels.

3.4. Gel fraction

Fig. 4 presents the gel fraction of the human hair-based and wool-based hydrogels and the pure PVA hydrogels prepared using EBI in the presence of PEI. The concentrations of keratin proteins (human hair
and wool) and blended PVA solutions were 5.0 wt.% and 5.0 wt.%, respectively. For the pure PVA solution (solution concentration ~5.0 wt.%), gelation did not occur until the EBI dose reached at 30 kGy, whereas the keratin protein solutions blended with PVA were successfully gelled at an even lower EBI dose, 10 kGy. As confirmed from SEM image, it was observed that the morphologies of the hydrogels derived from the human hair were coarse and rough, whereas the wool-based hydrogels densely compacted. When the keratin-based aqueous solution was cross-linked through the use of EB, the radicals were formed between the polymer chains through direct/indirect actions of radiation and solvent-derived radicals [56]. The wool-based hydrogels most likely formed more disulfide bonds than the human hair-based hydrogels. A hypothetical schematic is provided in Fig. 5. Human hair and wool are constituted largely of a three-dimensional mesh structure of keratin which is arranged of polypeptides made of different amino acids, inter- and intra-molecular bonding of disulfide cystine amino acid, and inter- and intra-molecular bonding of polar and non-polar acids. Two cysteine residues cross-linked again to form a disulfide bond through EBI; that is to say, sulfur side chains on the cysteine molecules after sulfitolysis [31,33] of human hair and wool were bonded to

![Fig. 2. SEM micrographs of freeze-dried hydrogels produced from (a) human hair and (b) wool solutions blended with PVA (keratin/PVA = 50/50, w/w) at a dose of 10 kGy and (c) pure PVA solution at a dose of 30 kGy in the presence of PEI (0.01 wt.%).](image)

![Fig. 3. FT-IR spectra of (a) human hair-based and (b) wool-based hydrogels and pure PVA hydrogels prepared using EBI in the presence of PEI.](image)

![Fig. 4. Effect of EBI dose on the gel fraction of human hair-based and wool-based hydrogels and pure PVA hydrogels prepared using EBI in the presence of PEI.](image)
another cysteine to form cys-S-S-cys by EBI. There was a slight shift of
the typical absorption peaks for the C=O and C-N-H bonds in the
FT-IR spectrum of the wool-based hydrogels, due to the intermolecular
interactions and cross-linking reactions between the polymer back-
bones in the wool and the amine groups in PEI or the -OH groups in
PVA, which could therefore affect the results of gel fraction, swelling
ratio and gel strength. The gel fraction of the human hair-based and
wool-based hydrogels was in the range of 70–84%. The gel fraction
increased as the EBI dose increased to 40 kGy and then saturated
(the gel fractions of human hair-based and wool-based hydrogels
were 84% and 79%, respectively), which indicated a constant degree of
cross-linking. The wool-based hydrogels exhibited a probable predom-
inination of scission reactions over cross-linking reactions above a dose of
40 kGy. Indeed, the addition of PEI may have effectively reduced the EBI
dose from 90 kGy to 10 kGy on the gelation of keratin proteins.

3.5. Degree of swelling

Fig. 6 shows the degree of swelling of human hair-based and wool-
based hydrogels and pure PVA hydrogels prepared using EBI in the
presence of PEI. The formation of the hydrogel indicated that a cross-
linking network was formed between the keratin proteins and PVA by
EBI. Both human hair and wool are keratin protein polymers, but they
have a variety of physical and covalent interactions between polymer
chains and the involved radical groups. Although the gel fraction was
greater in the human hair-based than in the wool-based hydrogels,
the swelling of the human hair-based hydrogels was unexpectedly
greater than the wool-based hydrogels. The degree of swelling of the
human hair-based and wool-based hydrogels increased as the EBI
dose increased to 40 kGy and then saturated to a constant degree of
swelling for the hair-based hydrogels and slightly decreased for the
wool-based hydrogels. For instance, the human hair-based hydrogels
exhibited a greater degree of swelling (~32.20 g/g) at 40 kGy, which
was presumably due to the considerable number of hydrophilic compo-
nents produced upon irradiation (chemical water absorption) and the
highly porous structures (physical water absorption) of human hair-
based hydrogels, which was confirmed by the SEM image in Fig. 2(a).
However, for the PVA hydrogels, the degree of swelling decreased as
the EBI dose increased.

3.6. Gel strength

The degree of cross-linking affects the mechanical properties and
water absorption ratio of hydrogels. Fig. 7 presents the gel strength of
the human hair-based and wool-based hydrogels and the pure PVA
hydrogels prepared using EBI in the presence of PEI. In particular, the
wool-based hydrogels exhibited significantly different gel strength
compared to hair-based and pure PVA at all EBI doses tested, with the
highest gel strength of 631 g · cm at the EBI dose of 20 kGy. The gel
strength of the hair-based hydrogels increased linearly as EBI dose in-
creased to 30 kGy and then remained constant (483 g · cm). In con-
trast to the keratin-based hydrogels, the PVA hydrogels exhibited a
dramatic increase in gel strength as the EBI dose increased, indicating
that a significant change in the hydrogel networks occurred. Mechanical
strength should be further developed before these hydrogels can be
used in practical applications and will be studied and reported in a
near future.
results in an enhanced absorption rate via a capillary force. This state of the keratin (human hair and wool)-based hydrogels, which is consistent with the results of the SEM analysis.

PVA hydrogels prepared using EBI in the presence of PEI. The EBI dose of the hydrogels and pure PVA hydrogels prepared using EBI in the presence of PEI. The EBI dose of the hydrogels was 10 kGy.

3.7. Kinetics of swelling

In practical applications, higher swelling capacities and higher swelling rate are required. Fig. 8 presents the swelling kinetics in water of the human hair-based and wool-based hydrogels and pure PVA hydrogels prepared using EBI in the presence of PEI. The freeze-dried keratin (human hair and wool)-based hydrogels absorbed distilled water rapidly (more than 26 g/g within 2000 min) and then reached a plateau, whereas the pure PVA hydrogels absorbed only approximately 9 g/g in the same absorption time. It is generally accepted that the swelling kinetics for the superabsorbent polymers is influenced by the pore size of the absorbents. Higher water uptakes are observed with polymers with smaller pore sizes [57]. However, the swelling behavior of keratin-based hydrogels was dominated by the larger pore size after 1000 min. Compared to pure PVA hydrogels, keratin-based hydrogels exhibited a higher absorption rate, a finding that suggests that human hair-based and wool-based hydrogels have potential practical applications because of their excellent swelling capacity and swelling kinetics. The higher swelling rate was most likely due to the highly porous and fibrous state of the keratin (human hair and wool)-based hydrogels, which results in an enhanced absorption rate via a capillary force. This finding is consistent with the results of the SEM analysis.

4. Conclusions

Biocompatible and highly porous keratin-based hydrogels were successfully prepared through EBI, and their synthesis significantly depended on several conditions, including the presence of PVA, concentration of keratin solution, EBI dose, and PEI additives. It was observed that adding a small amount (~0.01 wt.%) of PEI exerted a considerable effect on decreasing the EBI dose for the formation of the hydrogels from 90 kGy to 10 kGy. This finding may be attributed to covalent cross-linking between protein chains and physical cross-linking among the S-sulfo keratin main chains, oxygen groups in PVA aqueous solution, and amine groups of PEI. The resulting keratin-based hydrogels exhibited fibrous and highly porous morphologies, as confirmed by SEM analysis. The highest gel fraction and degree of swelling was achieved at the dose of 40 kGy. In addition, the degree of swelling and kinetics of swelling of the human hair-based hydrogels were superior to those of the wool-based and pure PVA hydrogels, presumably due to a considerable number of hydrophilic components produced upon irradiation (chemical water absorption) and the highly porous structures (physical water absorption) of the human hair-based hydrogels. These findings are in good agreement with the results of the SEM analysis. The resulting keratin-based hydrogels are expected to be more environmentally friendly than the PVA hydrogels.

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