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Radiation stability and modification of gelatin for biological and medical applications

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HIGHLIGHTS

- M_w of solid gelatin decreased by approximately7–10% with sterilization doses of 5–25 kGy.
- Hydrolysis rate of gelatin at 37 °C was decreased after radiation decomposition.
- Gelatin was radiation-crosslinked (RX) when irradiated in water solution.
- Simultaneous fabrication and sterilization of RX-gelatin hydrogel were performed.
- RX-gelatin hydrogel was stable for 7 days in water at 37 °C.

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ABSTRACT

Gelatin is used in various biological and medical fields, including drug delivery systems and tissue engineering. In the context of these applications, radiation sterilization of gelatin was evaluated in terms of radiation stability. The molecular weight of gelatin powder irradiated by electron beams (EB) was analyzed using gel permeation chromatography (GPC). We found that irradiation decomposed the gelatin and that the weight-averaged molar mass (M_w) decreased by approximately 7–10% with sterilization doses in the range of 5–25 kGy. Also, we found that the hydrolysis rate in body and cell culture environments (37 °C water) was affected by irradiation. Although gelatin powder underwent chain scission when irradiated, crosslinking was predominantly induced when the gelatin was irradiated in water solution. Radiation-crosslinked (RX) gelatin hydrogel was fabricated without using any crosslinkers. In this case, fabrication and radiation sterilization were performed simultaneously. Using gel fraction and GPC analysis of the eluted sol, it was determined that the RX-gelatin hydrogel was stable for 7 days in water at 37 °C. These results provide important data for evaluating the feasibility of biological and medical applications of gelatin and RX-gelatin hydrogel.

1. Introduction

Gelatin, a natural polymer derived from collagen, has many applications closely related to our daily lives, such as food products, pharmaceutical capsules, and cosmetics (Djagny et al., 2001). Gelatin is also used in advanced biological and medical technologies such as drug delivery systems and tissue engineering (Olsen et al., 2003; Young et al., 2005; Vlierberghe et al., 2011).

For medical and biological applications, sterilization of gelatin is necessary. Several sterilizing methods, such as heating, chemical treatment, and ionizing radiations, are available. Heat-sensitive materials such as gelatin are generally sterilized using ionizing radiation. Ionizing radiations such as γ-rays and electron beams (EB) provide an effective as well as bio and eco-friendly (without using toxic gases) method to sterilize materials (Dorpema, 1990). However, ionizing radiation induces chemical reactions in the irradiated materials. Therefore, it is important to evaluate radiation effects on gelatin for irradiation doses in the range of 5–25 kGy, which are normally used in the sterilization of food and medical products (Gopal, 1978; Diehl, 2002).

In literature, the effects of radiation on gelatin have been discussed in terms of the change in gelatin's viscosity. It has been reported that the viscosities of solutions containing γ-ray and EB-irradiated gelatin powder decrease in response to an irradiation dose (Vieira and Mastro, 2002). These results suggest the degradation of gelatin due to irradiation, but the topic has not been studied in detail.

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On the other hand, it has been found that in aqueous solution, under certain conditions, radiation induces three-dimensional networks in gelatin chains resulting in a crosslinked gelatin hydrogel (Tomoda and Tsuda, 1961; Terao et al., 2003; Bessho et al., 2007). When gelatin is immersed in hot water and cooled, it undergoes physical gelation owing to hydrogen and ionic bonding. Because the sol–gel transition occurs near the range of mammalian body temperatures, applications of gelatin in biological and medical fields are limited. In order to improve the mechanical and thermal properties of gelatin, crosslinkers such as formaldehyde and glutaraldehyde are generally used for processing (Olde-Damink et al., 1995). However, ionizing radiation can be used to induce crosslinking without such toxic reagents. For fabricating radiation-crosslinking (RX)-gelatin hydrogel, radiation doses equivalent to or higher than those used for sterilization are required. Therefore, the influence of radiation on RX-gelatin hydrogel should be examined.

In this study, the effects of radiation on both gelatin powder and RX-gelatin hydrogel were investigated. The molecular weight of EB-irradiated gelatin powder was determined using gel permeation chromatography (GPC).The effects of aging (preservation in water at 37 °C for 7 days) were also investigated in order to understand the stability of gelatin and RX-gelatin in the mammalian body's internal environment and in cell cultures. The stability of RX-gelatin hydrogel in water at 37 °C was investigated by determining the gel fraction and molecular weight of the eluted sol.

2. Experimental

2.1. EB irradiation

In this study, we used gelatin powder from porcine skin (Bio reagent, Type A, estimated molecular mass=50–100 kDa, from Sigma Aldrich, Inc., USA). Gelatin hydrogel samples were prepared by dissolving 10 wt% of gelatin in deionized water and mixing with a blending machine (ARE-250, THINKY). The solution formed gelatin gel at room temperature (23 °C). We irradiated the gelatin powder and the gel (thicknesses: approximately 2 mm) using EB in air with various irradiation doses. The acceleration energy and the beam current were 2 MeV and 2 mA (± 1%), respectively. The samples were uniformly irradiated by using a conveyor system. The radiation dose rate was 10 kGy/pass which was evaluated with a cellulose triacetate film dosimeter FTR-125 from Fujifilm, Japan (dose range: 5–300 kGy, uncertainty: ± 4%). The penetration range of 2 MeV EB is approximately 1.2 g/cm^2 (Seito et al., 2008).

2.2. GPC analysis

Molecular weight analysis was performed using TOSOH 8020 model Gel Permeation Chromatography (GPC) with a refractive index (RI) detector maintained at 45 °C. A phosphate buffer (0.1 M KH_2PO_4 and 0.1 M $Na_2HPO_4 \cdot 12H_2O$) from Wako Pure Chemical Industries (Japan) was used as the eluent at a flow rate of 1.0 ml/min. The temperature of the columns (TSK gel PWXL-M × 2 and PWXL2500) was maintained at 50 °C. The molecular weight of the samples was calculated using the calibration curve for polyethylene oxide (PEO) standards (from Fluka, Sigma-Aldrich, Japan). The samples were measured using GPC after being kept in the buffer at 37 °C for 0 (powders dissolved completely in 3 hours), 1, 4, and 7 days.

2.3. Gel content and swelling of irradiated gelatin hydrogel

The irradiated hydrogel samples were dried in a vacuum oven for 48 hat 30 °C and then weighed. The dried samples were immersed in deionized water at 37 °C to extract the sol fraction. Then, the swollen hydrogels, which consisted only of RX-gelatin, were filtered using a stainless steel 200 mesh and weighed. The obtained RX-gelatin hydrogel was dried in the vacuum oven at 30 °C for 48 h and weighed. The gel fraction (%) and swelling were calculated as

$$\text{Gel fraction}(\%) = (W_d/W_i) \times 100 \quad \text{and}$$

$$\text{Swelling} = (W_s - W_d)/W_d,$$

where W_i is the initial weight of the dried gel, W_s is the weight of the swollen hydrogel, and W_d is the weight of dried insoluble part (RX-gelatin hydrogel).

3. Results and discussion

3.1. Radiation-induced decomposition of gelatin powder

The effects of irradiations on gelatin powder were investigated using GPC. Gelatin powder was irradiated by EB with varying radiation doses (10, 20, 50, and 100 kGy) and dissolved in phosphate buffer eluent at 37 °C. Fig. 1(A) shows a gel permeation chromatogram of the control and EB-irradiated gelatin powders after they are completely dissolved in the buffer. The non-irradiated gelatin has two peaks at approximately 21.6 min and 20.6 min, which correspond to the α-chains (M_p=99.6 K) and β-chains (M_p=178.4 K), respectively. After irradiation, it was found that the peak intensity of the β-chains decreased, where as the peak intensity of the α-chains exhibited longer elution times with increasing irradiation dose (Fig. 1(A)). Fig. 1(B) summarizes the number average of molar mass (M_n), weight average of molar mass (M_w), and polydispersity index (PDI) of non-irradiated and irradiated gelatin. M_n and M_w for the non-irradiated gelatin were 83,000 and 157,000, respectively. It was found that the value of PDI was approximately constant (∼1.8) for all of the samples. These results clearly indicate the random chain scission of gelatin due to EB irradiation. M_w decreased by approximately 7–10% for sterilization doses in the range of 5–25 kGy.

The G-value of chain scission, $G(s)$, defined as the yield of chain-scission per 100 eV of absorbed energy, was determined using the GPC data. $G(s)$ is calculated using the Alexander–Charlesby–Ross equation

$$1/M_n = 1/M_{n0} + 1.04 \times 10^{-7} G(s) D,$$

where M_{n0} and M_n are the number averages of molar mass before and after irradiation, and D is radiation dose (in kGy). We determined that the $G(s)$ value of gelatin at an irradiation dose in the range of 0–100 kGy was approximately 0.84. This value is similar to the previously reported value of 0.98, which was estimated by osmometry of gamma-ray-irradiated gelatin (Friedberg and Hayden, 1966).

3.2. Hydrolysis of gelatin powder

GPC was also used to investigate the effect of aging on the molecular weight of gelatin in order to evaluate gelatin's durability for biological and medical applications. The gelatin samples were kept dissolved in the buffer at 37 °C for 7 days after EB irradiation. After 7 days, the gel permeation chromatogram is narrower (as shown in Fig. 2(A)) than the chromatogram shown in Fig. 1(A). The data demonstrate that gelatin samples are decomposed owing to both irradiation and preservation. The changes in M_w and PDI of gelatin with changes in irradiation dose and preservation time are summarized in Fig. 2(B). It was found that the PDI values remained stable at approximately 1.7. This result confirms that gelatin chains undergo random hydrolytic degradation (Scatchard et al., 1944). The decomposition rate of gelatin changed with irradiation dose.