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Comparison of the Stability of Poly-γ-Glutamate Hydrogels Prepared by UV and γ-Ray Irradiation

Sang-Joon Park[1], Hiroshi Uyama[2], Mi-Sun Kwak[1], and Moon-Hee Sung[1*]

[1]*Department of Bio and Fermentation Convergence Technology, BK21 PLUS Project, Kookmin University, Seoul 02707, Republic of Korea*
[2]*Department of Applied Chemistry, Graduate School of Engineering, Osaka University, Suita 565-0871, Japan*

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**Corresponding author*
Phone: +82-2-910-4808;
Fax: +82-2-910-5739;
E-mail: smoonhee@kookmin.ac.kr

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Poly-γ–glutamate (γ-PGA) has various applications due to its desirable characteristics in terms of safety and biodegradability. Previous studies have been conducted on γ-PGA hydrogels produced by γ-ray irradiation, but these hydrogels have proved unstable in solutions. This study was conducted to enable the γ-PGA hydrogel to maintain a stable form in solutions. The γ-PGA mixture for UV-irradiation was prepared with a cross-linker (N,N,N-trimethyl-3-[(2-methylacryloyl)amino]propan-1-aminium). Both γ-PGA hydrogels' characteristics, including stability in solutions, were examined. The UV-irradiated γ-PGA hydrogel maintained a stable form during the nine weeks of the study, but the γ-ray irradiated hydrogel dissolved after one week.

Keywords: Viscoelasticity, stability, Poly-γ–glutamate, UV-irradiated hydrogel, γ-ray irradiated hydrogel, *Bacillus* sp.

Over the centuries, hydrogels have been used as functional materials and applied in a variety of fields, such as in medical applications [1], for wound treatment [2] and in tissue engineering [3]. Depending on the polymer used, hydrogels can have a variety of desirable activities [4–6] in terms of strength, degradability, and stability. Recently, hydrogels have been made from natural polymers due to their biodegradability and biocompatibility, and a range of methods for making such hydrogels have been studied and applied [3, 7, 8].

γ-PGA is a negatively charged poly amino acid found in the sticky strings of cheonggukjang, a type of Korean fermented bean paste. Unlike conventional natural peptides, γ-PGA, which consists of γ-amide linkages of glutamate units, is biodegradable, safe for humans, and edible [7, 9–14]. γ-PGAs can be linked to each other through a cross-link between a α-amino group and an γ-carboxyl group to form a hydrogel. Numerous studies on γ-PGA hydrogels have already been conducted [8, 15, 16]. However, these previously developed γ-PGA hydrogels exhibited unstable form [15, 16] in solutions.

In this study, we prepared UV-irradiated γ-PGA hydrogels possessing a stable morphology in solutions with a high molecular weight of 3,000-kDa γ-PGA and reagents. The γ-PGA (potassium form of Mw = 3,000-kDa) was obtained from Bioleaders Corp. (Daejeon, Korea.). N,N,N-trimethyl-3-[(2-methylacryloyl)amino]propan-1-aminium (METH) was acquired from Wako (Japan). METH has previously been used to make hydrogels and soft contact lenses [17–19]. In addition, METH has been used in treatments for resistant acne and as a drug delivery system [20–22].

To make the γ-PGA hydrogels, γ-PGA solutions were prepared with the addition of reagents in the concentrations indicated. First, γ-PGA was dissolved in distilled water at 20 w/v % and stirred for 24 h at room temperature to arrive at a homogenous γ-PGA solution. After that, METH was added in order to obtain a molar ratio with glutamate (1:1) and stirred for 24 h at room temperature for the production of homogenous γ-PGA samples. The prepared samples were then irradiated with UV to prepare the hydrogels. The clearance from the UV irradiator was 15 cm, and the wavelength was 365 nm for 2 h, wherein the amount of irradiated energy was 50–70 mW/cm^2. The prepared high-viscosity solution was then transferred to a dish and air-dried at room temperature to prepare the UV-irradiated hydrogels. The γ-ray-irradiated hydrogels were

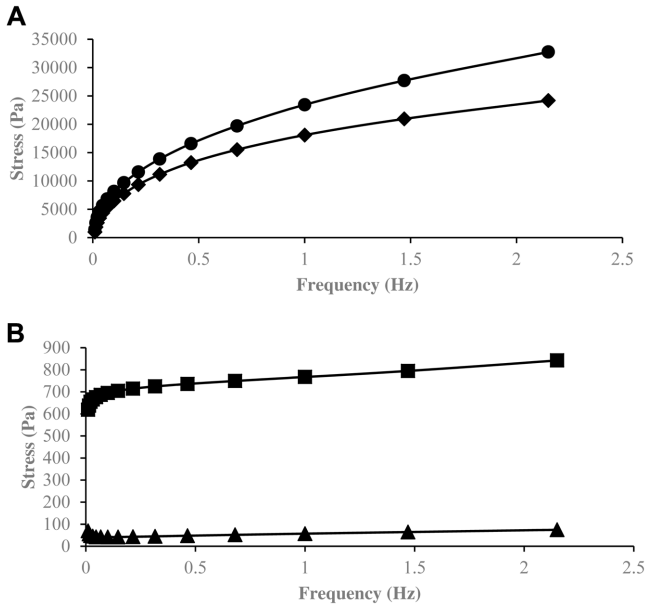

Fig. 1. Comparison of storage modulus (G′) and loss modulus (G″) between UV- and γ-ray-irradiated hydrogels.
(**A**) G′ and G″ of UV-irradiated hydrogel; (**B**) G′ and G″ of γ-ray-irradiated hydrogel. Symbol: (●), G′ of UV-irradiated hydrogel; (◆), G″ of UV-irradiated hydrogel; (■), G′ of γ-ray-irradiated hydrogel; (▲), G″ of γ-ray-irradiated hydrogel. Storage modulus and loss modulus of γ-PGA hydrogels.

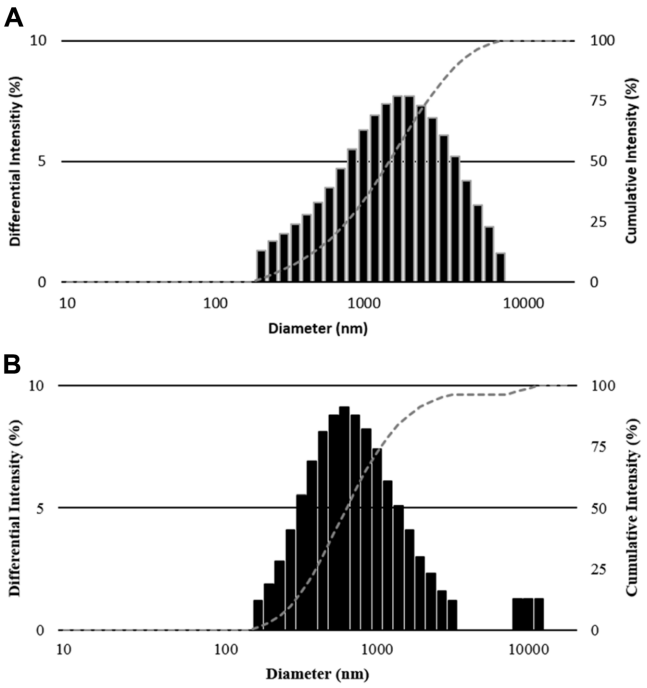

Fig. 2. The dynamic light scattering data for the particle size measurement of UV- and γ-ray-irradiated hydrogels.
(**A**) Dynamic light scattering data for UV-irradiated hydrogel; (**B**) Dynamic light scattering data for γ-ray-irradiated hydrogel.

prepared by γ-ray irradiation in accordance with the literature [8] and the γ-ray γ-PGA hydrogel obtained was lyophilized. The resulting lyophilized powder was dissolved in distilled water at 10% and used as the γ-ray-irradiated hydrogel in the experiment. Note that the UV-irradiated hydrogel was prepared in a different way because it was synthesized to compensate for the disadvantages of the existing γ-ray-irradiated hydrogel. UV causes the polymerization of γ-PGA. If polymerization occurs, the viscosity of the solution increases; thus, the storage modulus (G′) and loss modulus (G″) at this time were measured to visualize gelation.

The G′ and G″ of the prepared γ-PGA hydrogels were measured using a rheometer (Anton-Paar Co., Japan). The UV-irradiated hydrogel's G′ and G″ were 33,000 Pa and 24,000 Pa, and the γ-ray-irradiated hydrogel's G′ and G″ were 840 Pa and 75 Pa at 2.15 Hz, respectively. The UV-irradiated hydrogel's G′ and G″ were 39-fold higher and 320-fold higher than those of the γ-ray-irradiated hydrogel, respectively (Fig. 1).

To measure the particle size of each hydrogel, the UV-irradiated hydrogel and the γ-ray-irradiated hydrogel were lyophilized and pulverized to prepare hydrogel powder (powdered hydrogel). To obtain hydrogel particles, each prepared powder was dissolved in distilled water to prepare a 1% powder solution, which was treated with sonication. Via sonication, each hydrogel molecule falls apart. The particle diameter of each hydrogel was measured using an ELS-8000 (Otsuka Corp., Japan). The particle size of the UV-irradiated hydrogel and the γ-ray-irradiated hydrogel was 1024.5 ± 53.3 nm and 597.9 ± 37.2 nm, respectively (Fig. 2). These data suggest the particle formation from γ-PGA via the UV or γ-ray irradiation.

To observe particle formation, hydrogel powder was used. The particle formation of each hydrogel was observed using a scanning electron microscope (SEM) (Fig. 3). This difference may be due to the crosslinking method. In the UV irradiation, the crosslinking of METH takes place to cationic polyMETH, which is interacted with anionic PGA via ionic bonding to form the hydrogel. The reason why the flat cross-section image cannot be obtained for the UV-irradiated hydrogel via freeze drying may be related to this specific hydrogel structure; during the freeze drying, polyMETH and PGA are separated and coagulated, resulting in the irregular inner morphology.

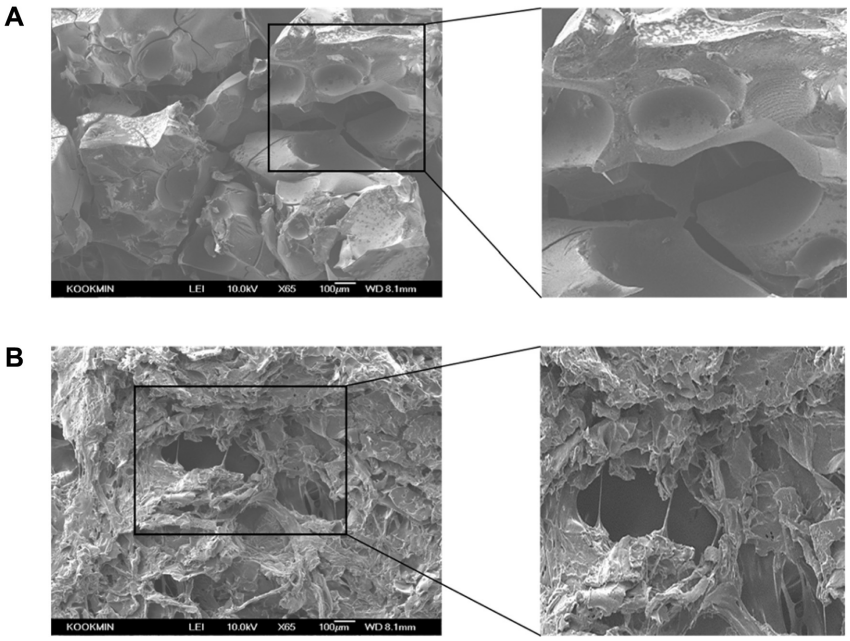

Fig. 3. SEM photographs of UV-irradiated hydrogel and γ-ray-irradiated hydrogel.
(**A**) UV-irradiated hydrogel (×65); (**B**) γ-ray-irradiated hydrogel (×65). SEM photographs of hydrogels.

Zeta potential was measured to estimate the number of carboxylate groups of γ-PGA hydrogels. The pH of each sample was adjusted to 4.8. Because the pKa of the γ-PGA was 4.8, the number of carboxylate groups in each sample was calculated using the zeta potential of a 1 μmol/ml γ-PGA solution (pH 4.8) as the control. The prepared γ-ray-irradiated hydrogel and UV-irradiated hydrogel were sonicated with deionized water, with the concentration of each hydrogel, per part of deionized water at that time being 10 w/v %. The zeta potential was measured using an ELS-8000 (Otsuka Crop., Japan). The zeta potential of the control was -40.20 ± 0.33 mV, while those of the γ-ray-irradiated hydrogels and the UV-irradiated hydrogels were -35.18 ± 1.72 mV and -29.30 ± 0.77 mV, respectively. To calculate the carboxylate groups in the prepared γ-PGA hydrogels, measured zeta potentials inclusive of the controls were used. The calculated number of carboxylate groups in the γ-ray-irradiated hydrogel and the UV-irradiated hydrogel were 14 μmol/g and 4.5 μmol/g, respectively. This suggests that the UV-irradiated hydrogel was cross-linked 3.1-fold more. The number of carboxylate groups in the hydrogel seems to be related to the G′ and G″ of the hydrogel [23].

To compare the degradation and stability of γ-PGA hydrogels in various solutions, the γ-PGA hydrogels were placed in a dish, and the solutions were poured in to immerse the hydrogels. The solutions used were distilled water, 20 mM potassium phosphate buffer (KPB) (pH 6.0), 0.9% NaCl, 1× phosphate-buffered saline (PBS), and simulated body fluid (SBF). Each buffer was chosen to mimic physiological conditions (pH, salt concentration, and salt composition). The γ-ray-irradiated hydrogel swelled in a way that was consistent with previous reporting [15, 16] and completely dissolved within one to two weeks, but the UV-irradiated hydrogel remained in the solutions (except for 1× PBS and 0.9% NaCl) for more than nine weeks. As a result, the UV-irradiated hydrogel showed a stable state in the solutions, especially in KPB (Fig. 4).

The prepared UV-irradiated hydrogel remained stable in solutions longer than the γ-ray-irradiated hydrogel, and exhibited a high G″. The UV-irradiated hydrogels displayed a higher zeta potential than the γ-ray-irradiated hydrogels. Each molecule of the UV-irradiated hydrogels was linked more closely together than those of the γ-ray-irradiated hydrogels. It is suggested that prior mechanical differences cause these discrepancies in properties. Recently, natural polymer hydrogels were used as bio 3D printing ink materials because of properties such as biocompatibility and stability [3, 24, 25]. The prepared UV-irradiated hydrogels also displayed these properties so they might have applications in a variety of areas such as biomaterial for artificial cartilage on the condition that enhancement in the mechanical

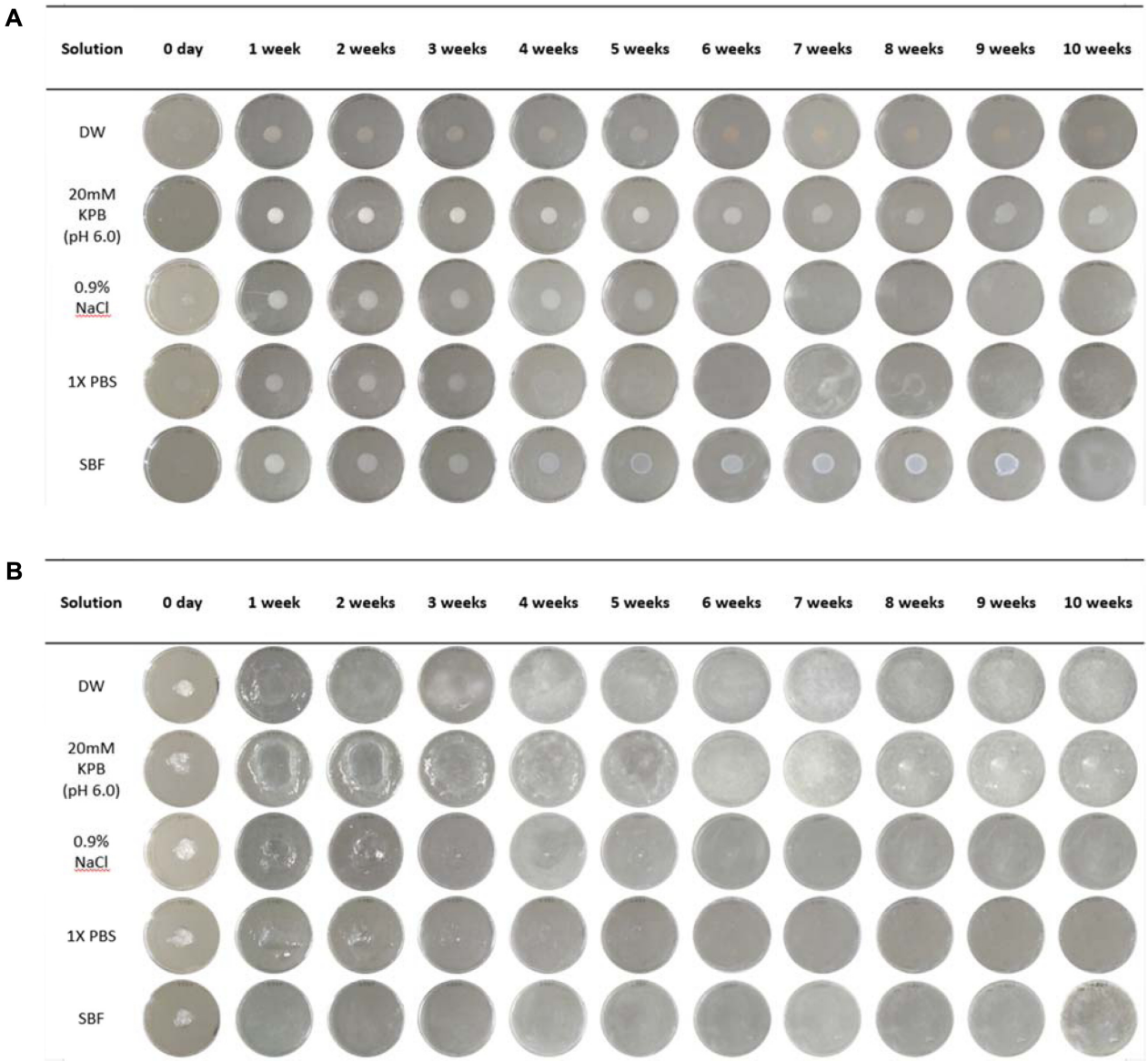

Fig. 4. Comparison of stability between UV- and γ-ray-irradiated hydrogels in various solutions.
(**A**) UV-irradiated hydrogel in various solutions during 10 weeks; (**B**) γ-ray-irradiated hydrogel in various solutions during 10 weeks. Stability comparison of γ-PGA hydrogels in various solutions. Used solutions were distilled water, 20 mM potassium phosphate buffer (KPB) (pH 6.0), 0.9% NaCl, 1X phosphate-buffered saline (PBS), and simulated body fluid (SBF). UV-irradiated hydrogel stable in SBF during 9 weeks.

properties of the UV-irradiated hydrogel takes place [3, 26]. Yet, the prepared UV-irradiated hydrogels' G′ and G″ still reached 110,000 Pa and 79,000 Pa when at 100 Hz, respectively.

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Conflict of Interest

The authors have no financial conflicts of interest to declare.

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