New Method of Injectable Hydrogels by Novel Photo-polymerization

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Introduction

From a clinical perspective, the use of injectable scaffolds is very attractive as it minimizes patient discomfort, risk of infection, scar formation, and treatment cost [1]. They are promising tissue engineering scaffold, especially for bone or soft tissue regeneration. In addition to serving as carriers for bioactive molecules, injectable scaffolds can also act as conduits for the guidance of tissue regeneration, tissue adhesives for healing, and injectable controlled release devices for local drug delivery [2]. Thermal gelation by using thermosensitive polymers is one of the most common strategies to make injectable systems due to the convenience of gelation and administration. For example, Pluronic hydrogels have been widely applied for the sustained delivery of various macromolecular drugs [3]. But, the physical nature of thermal gelation of Pluronic has limited its applications due to a fast dissolution after gelation [4]. To overcome this drawback, the secondary photo-polymerization after injection has been proposed. However, the existing injection method of photo-crosslinking thermosensitive hydrogels have intrinsic disadvantages such as the requirement of a special optical fiber and the discomfort of using it as well as the damage to normal cells and tissues around the injected polymer due to the direct exposure to UV irradiation [5].

In this study, utilizing the existence of the induction period in photo-polymerization, we propose a new injection method of photo-polymerizable, thermocrosslinking hydrogels made of di-acrylated Pluronic F127 (DA-PF127) without the use of optical fiber. First, the photo-polymerization of DA-PF 127 solution at molecular level is initiated by UV irradiation. And, this precursor solution is injected to a desired site before it becomes viscous by macroscopic gelation.

Experimental

Pluronic F127 (PF127) was derivatized to have acryl group at both hydroxyl ends using triethylamine and acryloyl chloride (98 % by ^{1}H-NMR). To a given concentration of di-acrylated Pluronic F127 (DA-PF127) solution in degassed 10 mM phosphate buffer (pH 7.4), 4-(2-hydroxyethoxy) phenyl-(2-hydroxy-2-propyl) ketone (Irgacure 2959), photo-initiator, dissolved in 70 % ethanol was added (1 % w/w). The proper UV irradiation condition that can make the injectable system was found for a given DA-PF127 concentration by observing the gelation kinetics of the solution using a rheometer (a parallel plate geometry made of acryl with a roughened surface) at 25℃. To validate the discovered injectable condition, UV was first irradiated in the determined proper condition to a DA-PF127 solution with initiator in a 5ml syringe at 25℃, and then the solution was injected via an 18 gauge needle into a rheometer sample holder at 37℃. The gelation was confirmed by monitoring change in the storage modulus.

In vitro protein release experiment was performed to compare the stability of the DA-PF127 hydrogels prepared by the present injectable method and the bare PF127 hydrogels. For each case, BSA was loaded into a sol state and the gelation was induced. Then, it was put into the release buffer. The released amount of BSA was assayed using a Micro-BCA kit.

Results and Discussion

For DA-PF127 solutions of 17, 13, and 9 % (w/w), the proper UV irradiation times to make injectable system after UV irradiation were found to be 2, 3 and 4 minutes, respectively, at 25℃; no change in the storage modulus (G') during the determined UV irradiation time, but after that, G' started to increase and became saturated (**Figure 1**). The frequency-independent G' at the final stage (**Inset of Figure 1**) verifies the crosslinked gel formation. By increasing the concentration of DA-PF127, the gelation rate became faster and G' became higher.

The determined injectable conditions were confirmed by running in vitro injectable gelation experiments; UV was first irradiated for 2, 3, and 4 minutes at 25℃ to 17, 13, 9 % (w/w) DA-PF127 solutions, and

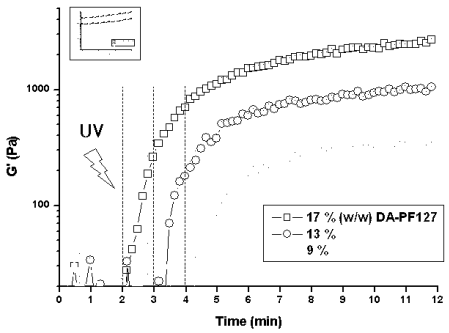

Figure 1. The gelation kinetics of 17, 13, 9% (w/w) DA-PF127 injectable hydrogels as a function of UV irradiation time for 2, 3, and 4 minutes, respectively, at 25℃

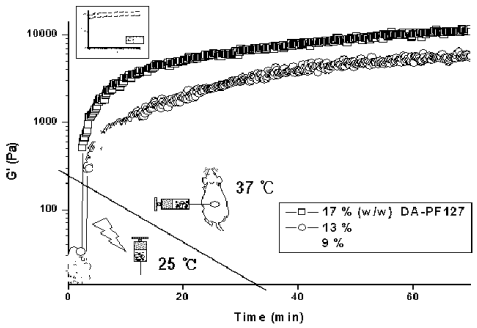

Figure 2. *In vitro* gelation kinetics of 17, 13, 9 % (w/w) DA-PF127 injectable hydrogels at 37℃ after UV irradiation for 2, 3, and 4 minutes, respectively, at 25℃

they were injected via a needle into a rheometer at 37℃. For all cases, G' increased rapidly and formed the gel states. The gelation rate and the final G' were higher for injectable gelation (37℃, **Figure 2**), compared to the results at 25℃ (**Figure 1**). *In vitro* BSA release studies also revealed the enhanced stability of the present system than bare Pluronic hydrogels; more sustained release of BAS was observed for the photo-crosslinked DA-PF127 prepared by the proposed method.

Conclusions

1. DA-PF127 solution can be injected and make a gel stated after UV irradiation without using the optical fiber.
2. The release rate of loaded proteins and the degradation time of hydrogels were enhanced by employing the proposed injectable system strategy.
3. This novel photo-polymerization method can be applicable to other polymers to make injectable scaffolds for biomedical applications.

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