A Study of Irradiated Silk Fibroin–Poly Vinyl Alcohol Hydrogel for Artificial Skin Substitutes

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Abstract

The artificial skin substitute was fabricated by blending PVA in silk fibroin solution at varied ratios. A silk fibroin composite solution with PVA was gelled using gamma irradiation. The result shows that the higher irradiation dose making a shorter time of gelation period. The maximum degree of swelling occurs at 25 kGy. The ATR-FT-IR shows a transformation of sol-gel transition between random-coil to beta-sheet structure at amide I (1700-1600 cm⁻¹). Therefore, structures of silk fibroin molecules are changed by gamma irradiation from 1650 to 1625 cm⁻¹ peak area. This transformation of silk fibroin structure is achieved at 40 kGy. The peak of random-coil (1650 cm⁻¹) is disappears and then the peak of bate-sheet are seen remarkably.

Key word: Silk fibroin, PVA, Hydrogel, Gamma ray irradiation, Artificial skin substitute

Introduction

Typically wound healing due to the skin loss is treated by surgery which removes the burned skin surface and quickly covered by the underlying tissue or skin grafting. Artificial skin substitute is invented as a three dimensional bilayer Extracellular Matrix (ECM) comprising a highly porous graft copolymer which degrades at a specific rate in the wound and regenerates the epidermis and dermis to replace a traditional method. The architecture of dermal scaffold is similar to a real skin. The advantage of hydrogel for artificial skin substitutes is high water content and good biocompatibility. It can be used as 3D ECM of skin substitution.

Silk fiber is consists of two main proteins; fibroin and sericin. Silk fibroin is a kind of natural protein polymers produced by Bombyx mori silkworms. Silk fibroin has unique properties such as tensile strength, biocompatibility, biodegradation, and support cell adhesion. Silk fibroin can be in various forms such as suture, film, fiber, sponge and hydrogel. They are shown to support cell adhesion, proliferation, differentiation in vitro and also tissue repair in vivo.

Materials and Experimental Procedures

A 0.5 degummed silk fibroin is dissolved in 10 ml of CaCl₂.H₂O: C₂H₅OH:H₂O by mole ratio 1:2:8 solution. The silk fibroin solution is dialyzed in distilled water for 2 days. The silk solution is then mixed with PVA in the wt ratio of 1:0.1, 1:0.2, 1:0.3, 1:0.5, 1:0.7 and 1:1 respectively. The optical density of samples is measured every 1 hour at 550 nm for degree of gelation.

The effect of gamma irradiation to gelation properties of silk fibroin and silk fibroin–PVA blended solution is carried out using silk fibroin-PVA blended at optimum condition from previous study with minimal gelation time, all samples are irradiated by gamma ray at 5, 10, 15, 20, 25, 30, 40, 50, 60, 70 kGy respectively, non-irradiation are used as a negative control. The optical density, FT-IR measurement for structure change (4000-400 cm⁻¹) and degree of swelling are monitored.

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Results and Discussion

Effect of Polyvinyl Alcohol (PVA) Addition in Silk Fibroin Solution for Gelling Properties

The optical density change at 550 nm, silk fibroin-PVA blended samples were monitored throughout the gelation periods. These results show that the higher PVA content shortening the gelation period, as seen in Figure 1. The polyvinyl alcohol (PVA) has impacted directly on gelation by increasing rate of crosslink between silk structure and PVA structure.

![Figure 1](image.png)

Figure 1. Gelation time of silk-PVA composite was measured by Optical density at 550 nm.

Effect of Gamma Irradiation to Gelation Properties of Silk Fibroin and Silk Fibroin–PVA Blended Solution

Silk fibroin–PVA composites at varied ratio were irradiated by gamma irradiation. The optical density of silk solution was monitored after the irradiation as shown in Figure 2. Pure silk solution without PVA was gelled after 16 hours of lowest irradiation dose (5 kGy). The increasing of irradiation dose the gelation process becomes faster up to 30 kGy, where the solution gelled immediately. These results depict that the irradiation does at 30 kGy can induce pure silk solution to gel at the shortest time. The variation of irradiation dose to silk fibroin-PVA blended shows that the increasing gamma ray intensity or PVA content can induce the rate of gelation

![Figure 2](image.png)

Figure 2. Silk fibroin sol-gel transitions at different doses was measured at 550 nm.

![Figure 3](image.png)

Figure 3. Optical density of irradiated silk-PVA blended at (a) 5 kGy, (b) 10 kGy, (c) 20 kGy, (d) 30 kGy respectively.

The prepared silk fibroin hydrogels were completely dissolved in distilled water for 48 hours. The water absorption in silk fibroin-PVA hydrogel started at 15 kGy irradiation and gradually
increase when samples are irradiated at 25 kGy which exhibited the maximum water uptake level, this water uptake level decreased and almost stable when irradiation intensity increase to stability (as shown in Figure 4), this indicating that the increment of the irradiation dose over 25 kGy may decrease the gap the crosslinked structures causing the poor water absorption.

Figure 4. Degree of swelling of silk fibroin-PVA hydrogel.

The structural analyses of composites were detected by ATR-FTIR as shown in Figure 5. The spectral changes of pure silk solution and composite solution were observed both irradiation and non-irradiation condition. The peak band of random-coil is 1650 cm⁻¹, the peak beta-sheet structure is 1660-1695 and 1625-1620 cm⁻¹ (amide I). The non-irradiated samples show random-coil peak which is higher than beta-sheet peak as seen in Figure 5 (a). The change of elevated doses of silk fibroin with and without PVA content (Figure 5 (b)) are indicated the shift of peak position of random-coil to beta-sheet remarkably. The 40 and 50 kGy are observed obviously for a completely shift to beta-sheet structure.

Figure 5. ATR-FTIR spectra (a) pure silk fibroin (b) silk fibroin-PVA 1:0.05, (c) 1:0.1, (d) 1:0.2 respectively.
References

