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Enzymatic Degradation Properties of Silk Fibroin Film

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Abstract

The degradation behavior of silk fibroin biomaterials in human body is definitely vital for the growth of tissues. Therefore, an investigation to regulate the degradation behaviors of silk fibroin films by changing the degree of cross-linking is presented in this paper. The in-vitro experiments in the simulated human body environment showed that the degradation rate of cross-linked silk fibroin films was inversely proportional to the degree of cross-linking. After degradation, the ratio of crystalline part in the SF films increased. This approach would provide a new direction in controlling the degradation time by cross-linking the silk fibroin for specific tissue engineering application.

Keywords: Silk fibroin; Films; Cross-linking; Degradation

1 Introduction

Silk Fibroin (SF) is a natural protein produced by the domestic silkworm, *Bombyx mori*. The amino acid composition of silk fibroin from *Bombyx mori* consists primarily of glycine, alanine and serine [1]. The three simple amino acids form the crystalline regions of silk fibroin, while the amino acids with bulky and polar side chains form the amorphous regions [2]. The silk polymorphs include silk I, silk II and an air/water assembled interfacial silk III [1, 3].

Due to the unique chemical and mechanical properties and biocompatibility, *Bombyx mori* silk fibroin materials have been invested as biomaterials for years. An ideal biomaterial is one that is non-immunogenic, has non-toxicity, and biocompatible, which can be functionalized with bioactive proteins and chemicals [4]. Another key factor is that the material should be biodegradable, and the degradation products should be non-toxic, easily metabolized and cleared from the body [5].

The silk has been used for manufacturing surgical sutures for one century [6]. In recent years, regenerated silk fibroin have been used extensively in biomedical applications, such as burn-wound dressings [7], drug delivery matrices [8], vascular prostheses and structural implants [9, 10], ligaments [11], bone [12, 13], cartilage [13], nets [14], and so on. These results show that silk fibroin is one of the most precious raw materials for being used as biomaterials.

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Recently, based on the importance of degradation for biomaterial applications in tissue engineering, the degradation of SF materials has been well studied [2, 6, 15-17, 19]. Silk is defined by United States Pharmacopeia as non-degradable for its negligible tensile strength loss in vivo [4]. However, according to the literature, as a protein, silk can be gradually digested by proteolytic enzymes and absorbed in vivo over a long time [16]. The rate of degradation depends on the properties of the material itself, as well as the chemical and biochemical environment of the site of implantation [17]. These results highlight that silk fibroin biomaterials are biodegradable, and the degradation behaviors could be regulated. However, the relationship between structure, processing, and degradability is not clear.

The goal of the present study is to make a preliminary research of the relationship between the degree of cross-linking of the SF films and the degradation behavior in vitro, controlling over the rate of degradation. Chemical, physical, and morphological features of the biodegraded SF films were examined by means of XRD, SEM.

2 Materials and Methods

2.1 Preparation of Silk Fibroin Films

The *Bombyx mori* silk samples were treated three times with Na_2CO_3 solution respectively to remove sericin. Then they were rinsed and air dried. The pure silk fibroin fibers were dissolved with triad solvent $CaCl_2/CH_3CH_2OH/H_2O$ (mole ratio=1:2:8) through stirring. The prepared solution was purified by dialyzed against distilled water for 4 days, to obtain silk fibroin solution. The genipin (GP) powder was added to the silk fibroin solution with constant stirring for 20 minutes, according to the percentages (0%, 5%, 10%, 20%, 30%) of solute mass. The mixed solution was incubated at 37℃ for 12 hrs. Then 40 ml solution was cast on a polyethylene plate at 60℃ for 2 hrs.

2.2 Evaluation of Cross-linking Index

The degree of cross-linking was measured using ninhydrin assay [18]. Ninhydrin (2, 2-dihydroxy-1, 3-indanedione) is widely used as a chemical reagent for the colorimetric determination of amino acids. It reacts with free α-amino groups to produce an aldehyde, carbon dioxide, and reduced ninhydrin through reaction. Ninhydrin assay was used to determine the amount of free amino groups of each test sample. The silk fibroin films with a constant mass (0.05 g) were incubated in 1.5 ml distilled water for 1 hour, and then 450 μL 0.1% ninhydrin solution was added to the sample. The test sample with a ninhydrin solution was heated for 20 min at 100℃. Then the optical absorbance at 570 nm (the wavelength of the blue-purple color) of the solution was recorded. Use 200 μg/ml glycine solutions as standard. The cross-linking index was defined as (1).

$$\text{Cross-linking index (\%)}=[(\text{free amino groups before cross-linking})-(\text{free amino groups after cross-linking})]/[\text{free amino groups before cross-linking}] \quad (1)$$