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Protein-based wound dressings

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KEYWORDS
chronic wound, silk fibroin, keratin, elastase, inhibitor protein

ABSTRACT

The unique properties of silk fibroin were combined with keratin to develop new wound dressing materials. Silk fibroin/Keratin (SF/K) films were prepared to reduce high levels of elastase found on chronic wounds. This improved biological function was achieved by the incorporation of a small peptide synthesized based on the reactive-site loop of the Bowman-Birk Inhibitor (BBI) protein. *In vitro* degradation and release were evaluated using porcine pancreatic elastase (PPE) solution as a model of wound exudate. It was found that biological degradation and release rate are highly dependent on film composition. Furthermore, the level of PPE activity can be tuned by changing the film composition showing therefore, an innovative way of controlling the elastase-antielastase imbalance found on chronic wounds.

INTRODUCTION

Wound healing is a specific biological process related to the general phenomenon of growth and tissue regeneration. Wound healing progresses through a series of interdependent and overlapping stages. The interruption of the orderly sequence of events during the healing process results in chronic wounds (Martin, 1997). Excessive amounts of exudates are present in these types of wounds causing maceration of healthy skin tissue around the wound, inhibiting the healing. In addition, exudate from chronic wounds contains high levels of tissue destructive protease enzymes namely, polymorphonuclear (PMN) elastase (Enoch and Leaper, 2008). The excessive action of elastase leads to reduce amounts of growth factors and endogenous proteinase inhibitors, causing the cleavage of collagen, elastin and fibronectin, and consequently the destruction of extracellular matrix (Chufa, et al., 1999, Dorne and Benedict, 1999). As a result, there has been considerable interest in the design of inhibitors that restore the elastase – antielastase imbalance. Bowman-Birk inhibitors (BBIs) are small plant proteins. They have a symmetrical structure of two tricyclic domains each containing and independent binding loop (Chen, et al., 1992). The inhibition of serine proteinases is often mediated by these binding regions. Small peptides mimicking the reactive-site loop of BBIs protein have shown to retain much of the inhibitory activity of the complete protein (Leatherbarrow, 2001). This study focus on the development of wound dressings with the ability to control elastase activity. Silk fibroin and keratin blends were used to incorporate a synthetic BBI peptide. The excellent properties of silk fibroin such as high mechanical strength, low degradability and biocompatibility were combined with keratin protein in order to modulate the physical and bifunctional properties of the final material to fulfill the wound healing needs.

RESULTS

In vitro degradation of SF/K films was determined by incubating protein films for several days in the presence of porcine pancreatic elastase (PPE). It can be observed (Figure 1) that pure SF films present a low degradation rate that remains constant over the time exposure to elastase. In the blends, it can be observed that the weight loss obtained is a function of keratin content present in the film. From our results the maximum keratin amount that promotes a constant degradation is 40%. The films kept in buffer solution showed little or no degradation in 14 days.

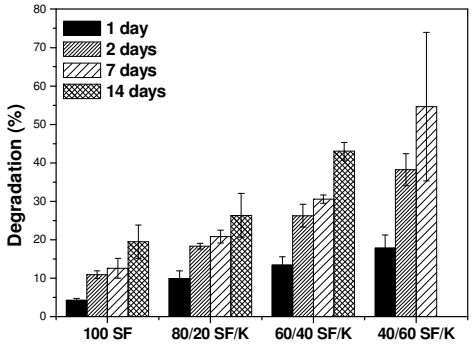

Figure 1: In vitro degradation of protein films incubated with 0.1 U/mL of elastase solution at 37 ºC.

To determine the inhibitory activity of the peptide, increasing peptide amounts were incubated with elastase solution at 37 ºC. From the results obtained on table 1 it can be seen that for high peptide concentrations PPE activity rapidly decreases, suggesting its ability to act as an elastase inhibitor. For lower peptide concentrations the decrease in half-life time of PPE is not so pronounced. It can be concluded that the decrease in PPE activity is dependent of peptide concentration.

Table 1: Hal-life time of PPE activity

Sample	$t_{1/2}$ (h)
PPE	28.3 ± 3.7
PPE + 20 µM	15.3 ± 2.1
PPE + 40 µM	11.6 ± 2.3
PPE + 60 µM	5.3 ± 1.2
PPE + 80 µM	3.8 ± 1.1

When the peptide was incorporated on the films there is an increases of the half-life time of when compared with the peptide alone (Table 1). This result indicates the ability of the SF/K films to act as elastase inhibitors wound dressings.

Table 1: Hal-life time of PPE activity after 24 h of incubation with different inhibitor peptide concentrations at room temperature

Sample	$t_{1/2}$ (h)
PPE	28.3 ± 3.7
100SF	22.4 ± 4.2
80/20 SF/K	17.5 ± 3.3
60/40 SF/K	14.1 ± 2.1
40/60 SF/K	8.1 ± 1.1

Cytotoxic evaluation was performed by seeding the cells on the films. The results showed a time dependent increase of cell metabolic activity (Figure 2) that suggest an increase of cell proliferation. At 48 h (Figure 3), cells exhibited an elongated morphology with fusiform fibroblastic appearance already at confluence, as on the control (TCPS). involving the use of solvent systems will not cause

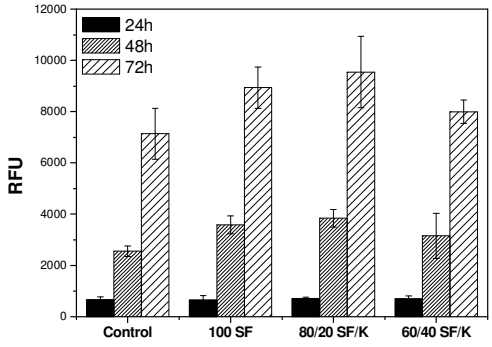

Figure 2: NIH 3T3 cell proliferation on SF/K films.

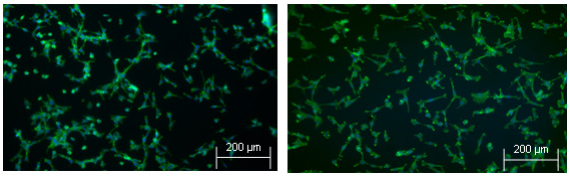

Figure 3: Fluorescent labelling of NIH 3T3 cells cultured for 48 h on TCPS (A) and SF/K film (B).

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