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# Biofunctionalized electrospun silk mats as a topical bioactive dressing for accelerated wound healing

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**Abstract**

Materials able to deliver topically bioactive molecules represent a new generation of biomaterials. In this article, we describe the use of silk mats, made of electrospun nanoscale silk fibers containing epidermal growth factor (EGF), for the promotion of wound healing processes. In our experiments, we demonstrated that EGF is incorporated into the silk mats and slowly released in a time-dependent manner (25% EGF release in 170 h). We tested these materials using a new model of wounded human skin-equivalents displaying the same structure as human skin and able to heal using the same molecular and cellular mechanisms found in vivo. This human three-dimensional model allows us to demonstrate that the biofunctionalized silk mats, when placed on the wounds as a dressing, aid the healing by increasing the time of wound closure by the epidermal tongue by 90%. The preservation of the structure of the mats during the healing period as demonstrated by electronic microscopy, the biological action of the dressing, as well as the biocompatibility of the silk demonstrate that this biomaterial is a new and very promising material for medical applications, especially for patients suffering from chronic wounds.


*Keywords:* Wound dressing; Wound healing; EGF; Growth factors; Silk fibers

## 1. Introduction

For several decades, a number of research groups have been working on strategies to promote the process of wound healing [1–3]. Indeed, the wound healing process is complex and involves the interactions of many different types of cells and matrix components to establish a provisional tissue and eventually a complete regenerated epidermis [1–3]. Among the different steps involved in wound healing, the closure of the wounded area by the epithelial cells is one of the most important as it restores an intact epidermal barrier and protects the underlying tissue. Reepithelialization involves the controlled migration of keratinocytes after their division at the wound edges (epithelial tongue) [4]. In some pathological conditions, such as diabetes, reepithelialization never occurs, leading to the development of acute or chronic non-healing wounds. To promote healing, wound dressings should meet several criteria: (1) biocompatibility, (2) prevent dehydration of the wound and retain a favorable moist environment, (3) physically protect the wound against dust and bacteria, (4) allow gas exchange, and (5) promote epithelization by delivering specific, active molecules.

Recently, we have been exploring a biological protein, silk fibroin from the *Bombyx mori* silkworm, for wound dressings. Silks represent a new family of advanced biomaterials due to their unique attributes of high mechanical strength, excellent biocompatibility, and the ability to control the structural and morphological features of the silk proteins. It has been established as an invaluable material

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in the field of biomedical engineering ranging from skin, bone, and vascular grafts [5–9]. In addition, we have successfully developed a method to electrospin silk through a completely aqueous process to generate useful new biomaterials [9]. Indeed, electrospinning is a simple, versatile, and useful technique for fabricating nanofibrous membranes from a rich variety of functional materials [10–12]. The porous, nanofibrous structured electrospun membranes create favorable properties as wound dressings including: controlled evaporative water loss, excellent oxygen permeability, promotion of fluid drainage, and inhibition of exogenous microorganism invasion due to their ultra-fine pores. While a significant number of natural and synthetic materials have been electrospun to form wound dressings, challenges remain in terms of biocompatibility, mechanical properties, and overall functional performance. Moreover, the all water-based electrospinning approach has further permitted the incorporation during the process of labile cell signaling factors that retain their biological function (bio-functionalization), as it has recently been demonstrated in bone formation with incorporation of bone morphogenetic protein-2 (BMP-2) [13].

Here, we present the functionalization of silk mats to enhance wound healing using epidermal growth factor (EGF). Our choice was motivated by the important role played by EGF in the wound healing process, especially the stimulation of proliferation and migration of keratinocytes [14–16]. EGF has high affinity receptors expressed in both fibroblasts and keratinocytes and has been shown to accelerate wound healing in vivo [17,18]. It has been demonstrated that the first 5 days after injury are the most critical during which maximal differences are seen between EGF treated and untreated wounds. EGF application after this period produces no significant improvement over controls, since by this time reepithelialization has already occurred in both groups [19]. Due to its relatively short half life of about 1 h [19], loss of occupied receptors through turnover, and a lag time of 8–12 h to commit cells to DNA synthesis [18], it is necessary to apply EGF frequently to a wound to maintain effective local concentration during the critical period of initial wound healing [19]. Therefore, topical applications of this molecule, such as a dressing applied on top of the wound, could be an easy but powerful way to locally deliver EGF and to protect the tissue during the reconstruction phase.

In the present report, we have used electrospun silk mats that were functionalized by adding a growth factor (EGF) during the electrospinning process. The release rate of the EGF from electrospun silk mats was evaluated by ELISA immunodetection over a 6 day period. These mats were then tested on top of a wounded human skin-equivalent in order to measure the wound healing rate in tissues that accurately mimic the human wound response. The use of EGF-charged silk mats increased the rate of wound closure by more than 3.5-fold when compared to the silk dressing without EGF. Moreover, the mats were observed before and after they were used on wounds by electron microscopy to demonstrate the conservation of the material integrity during the complete healing process. Taken together, our results demonstrate that this new material has tremendous potential for novel therapeutic bioapplications especially since these silk mats present many properties that would be ideal for creating a new generation of biologically active wound dressings for the acceleration of non-healing chronic wounds.

## 2. Materials and methods

### 2.1. Preparation of silk mats

Cocoons of *B. mori* silkworm silk were kindly supplied by M. Tsukada, Institute of Sericulture, Tsukuba, Japan. A silk fibroin aqueous solution was prepared as previously described [13]. Briefly, cocoons were boiled for 30 min in an aqueous solution of 0.02 M $Na_2CO_3$, and then rinsed thoroughly with distilled water to extract the glue-like sericin proteins. The extracted fibroin was then dissolved in 9.3 M LiBr solution at 60 °C for 4 h, yielding a 20% (wt./v) aqueous solution. This solution was dialyzed against distilled water using Slide-a-Lyzer dialysis cassettes (MWCO 3500, Pierce) at room temperature for 3 days to remove the salt. The dialysate was centrifuged two times, each at −5 °C to 10 °C for 20 min, to remove impurities and aggregates. The final concentration of the silk fibroin aqueous solution was approximately 8% (wt./v). To fabricate electrospun membranes, the silk solution was then blended with 5.0 wt.% polyethylene oxide (PEO) (900,000 g $mol^{-1}$) to increase the solution viscosity and stabilize the jet during the electrospinning process to produce the 7.5 wt.% silk/PEO solution for spinning, as we have previously reported [13]. EGF solution (human EGF, R&D Systems, Minneapolis, MN) was mixed well to generate a final concentration of 10 μg EGF/ml silk fibroin.

Electrospinning was performed with a steel syringe needle with an inner diameter of 1.5 mm mounted on an adjustable, electrically insulated stand as described in Fig. 1. The syringe needle was maintained at a high electric potential for electrospinning and mounted in the parallel plate geometry. A constant volume flow rate of 0.02 ml $min^{-1}$ was maintained using a syringe pump. The voltage was kept at 10.0–11.0 kV and the distance between the syringe needle and the grounded collection plate was 15.0–21.0 cm. The electrospun mats were collected on a collection plate covered with aluminum foil followed by methanol treatment to obtain water insoluble silk mats. The average thickness of the dressings used in this study was around 300 μm. Variations in thickness for each fabrication did occur due to fluctuations in voltage, humidity, and the conductivity of the collection target. This is the limitation of electrospinning which could be minimized by better control of the parameters. The dressing samples in this study were equivalent in thickness as they were prepared from the same piece of electrospun mat. All the pieces were kept at 4 °C and used less than one week after spinning. Before putting the mats on the wounds they were