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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

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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 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 sterilized by a quick spray of ethanol 70% and let dry under sterile atmosphere.

2.2. Cells

Human dermal fibroblasts (HDF) used for skin-equivalent cultures and scratch wound healing assays were derived from newborn foreskins using a combination of collagenase and Trypsin/EDTA as previously described [20]. HDFs were grown in DMEM (Gibco, BRL) supplemented with 10% fetal bovine serum (Hyclone Laboratories), penicillin–streptomycin (1.12 unit ml^{-1}, Sigma), and HEPES (Sigma). Cells were usually seeded at a density 5 × 104 cells ml^{-1} (~10% confluent) for extended cell passage and cultures were sequentially passaged when cell density reached confluence. Normal human keratinocytes (NHK) were isolated from newborn foreskin by the method of Rheinwald and Green [21] and maintained in keratinocyte medium described by Wu et al. [22]. Cultures were established through trypsinization of foreskin fragments and grown on irradiated 3T3 fibroblasts. 3T3 cells were maintained in DMEM (Gibco, BRL) containing 10% bovine calf serum (Hyclone Laboratories). All cells were grown at 37 °C in 7.5% CO_2.

2.3. Fabrication of human skin-equivalent wound healing model

Skin-equivalent cultures were prepared and wounded in vitro as previously described by Garlick and Taichman [23]. Briefly, to construct the collagen matrix, HDF cells were added

to neutralized Type I collagen (Organogenesis, Canton, MA) to a final concentration of 2.5 × 104 cells ml^{-1}; 3 ml of this mixture was added to each 35 mm well insert of a 6-well plate and incubated for 7 days in media containing DMEM and 10% fetal calf serum until the collagen gel showed no further shrinkage. At this time, of 5 × 105 NHKs were plated directly on a raised, mesa-like area is seen in the center of the contracted collagen gel. Cultures were submerged in low calcium epidermal growth media for 2 days, submerged for 2 days in normal calcium epidermal growth media and raised to the air–liquid interface by feeding from below at 37 °C in 7.5% CO_2. Skin-equivalent cultures were wounded 7 days after keratinocytes were seeded onto the collagen matrix. One week before cultures were to be wounded, an additional collagen matrix was fabricated as described above. This was used as the substrate onto which the wounded skin-equivalents were transferred. To generate wounds, the skin-equivalent culture was removed from the insert membrane using 1.5 cm punch, and a 4 mm punch was used to incise the epidermis and collagen matrix. Wounded skin-equivalents were transferred onto the 7-day-old collagen matrix. Wounded cultures were maintained at the air–liquid interface for 24 h, and 48 h at 37 °C in 7.5% CO_2 to monitor reepithelialization.

2.4. Histology analysis

For routine light microscopy, skin-equivalent cultures were fixed in 4% neutral buffered formalin, embedded in paraffin, and serially sectioned at 8 μm. Sections were stained with hematoxylin and eosin, visualized and measured using a Nikon eclipse 80i microscope equipped with Diagnostic Instruments SPOT RT Camera.

2.5. ELISA test assay

The amount of EGF released was determined by punching the silk mat containing EGF with a 1.5 cm punch, the disk of silk was then placed in PBS, 100 μl of sample were taken at different times (1, 2, 3, 4, 5, 6, 7 h, and 1, 2, 4, 6, 7 days). For the in vitro experiments, a 1.5 cm punch of silk mats containing EGF was put directly on top of the wound. In this case, 100 μl of medium surrounding the well with the wound were taken at different times and replaced by fresh medium. The samples were then frozen and kept at −20 °C until assayed for the cytokine levels using ELISA kits specific for human EGF (Quantikine, R&D Systems, Minneapolis, MN). Serial dilutions were performed to determine cytokine concentrations by comparison with the standard according to the manufacturer's instruction. The amount of EGF released after a week in PBS was chosen to be set at 100%, that means that after a week, the quantity of EGF that has been released in vitro in PBS represents 100% of the EGF that can be released from a silk mat of 1.5 cm diameter.

Then, we measured the amount of EGF released when a silk mat of 1.5 cm diameter was put on top of the wound. Samples of the medium surrounding the wound were taken at different times (1, 2, 3, 4, 5, 6, 7, 24, 48, 96, 144 and 168 h) and the amount of EGF present in these sample was determined by ELISA. This amount was compared to the amount of EGF released in PBS after a one week period and we obtained the percentage of EGF released when the silk mats were put in contact with the wound.

2.6. Scanning electron microscopy (SEM)

For electrospun silk mats, the materials were sputter-coated with gold/palladium using a Polaron SC502 Sputter Coater (Fison Instruments, UK). Specimens were then examined using an ISI-DS-130 SEM (Avon, CT) at 15 kV.

2.7. Statistical analyzes

Statistical comparisons were performed using XLSTAT software and were based on an analysis of variance (ANOVA) and student's *t*-test for pairwise comparisons. In our experiments, $p < 0.01$ was considered significant).

3. Results

3.1. Preparation of silk mats

Silk fibroin from the silkworm *B. mori* consists of about 60% glycine and alanine repeats. The fibroin chain consists of two basic polypeptide sequences, crystalline and less ordered polypeptides that alternate regularly. The basic sequence of the "crystalline" polypeptides is –(Ala–Gly)n– that adopts a β-sheet structure, whereas the "less ordered" polypeptides contain additional amino acids, in particular, tyrosine and valine as well as acidic and basic amino acids [24]. The presence of polyethylene oxide in the fibroin solution causes an increase in the viscosity of the solution prior to the electrospinning process. The silk solution is injected through a needle and submitted to a high potential difference that will create a jet of silk solution. This jet is unstable and thus will solidify before coming in contact with the collector. The electrospinning apparatus and process is depicted in Fig. 1. The settings were identical for silk solutions with or without the growth factor EGF. The presence of EGF does not lead to any changes in the electrospinning process, the EGF dissolves in the silk fibroin/PEO solution, and no mixing problems have been encountered when adding the growth factor.

The electrospinning process leads to the formation of silk fibers. Fig. 2 shows scanning electron microscopy images of electrospun fibers containing the growth factor EGF. The structure of the silk mats consists of randomly oriented fibers of uniform diameter. The fibers are interconnected, creating a three-dimensional porous network. This network is a solid mat that can be easily removed from the collector and cut to size to be placed on top of the in vitro wounds.

3.2. Wound healing model

Normal wound healing begins with proliferation and migration of keratinocytes from the wound edge and is followed by proliferation of dermal fibroblasts in the neighborhood of the wound. To follow these events step by steps an in vitro three-dimensional model of wound healing was used. Fig. 3 displays human skin-equivalents (HSEs) that have been adapted to study the appearance of wounded keratinocytes and tissue phenotype during reepithelialization. In our experiments, the silk mats with or without the growth factor were punched to obtain disks of 1.5 cm in diameter. These thin mats were then put directly on top of the wound (Fig. 3c).

3.3. EGF release

To evaluate the stability of EGF, the immunoreactivity of EGF in the electrospun mats was determined by ELISA. For these experiments, we used silk mats of the same weight (0.65 ± 0.02 mg) and calculated that the initial EGF amount in each sample is 86.7 ± 2.67 μg. By considering this amount of EGF as 100% and normalizing the amount of EGF released in PBS we obtain a release profile of EGF from silk mats that have been put on top of the wound model in vitro depicted in Fig. 4. Though we know the exact amount of EGF added to the fibroin silk/PEO solution, it is not possible to ensure that the concentration of EGF stays the same during the electrospinning process, or to be certain that the electrospinning step is not inactivating a certain amount of the protein. We thus decided to determine the amount of EGF that can be effectively released from the silk mats. These silk mats were put in PBS and the amount of EGF released was quantified over time. The average amount released at 37 °C was defined as

the maximum quantity of EGF that can be released from a silk mat of 1.5 cm in diameter. This amount was then compared to the amount of EGF released when the silk mats were put on top of the wound. The profile of the percentage of EGF released shows a burst during the first day. After this burst effect, the release profile is a stable and increases regularly over a 6 day period. After one week of contact with the wound, the amount of EGF released from the silk mats is 24.7 ± 0.8%. This result can be explained by the fact that the two experiments are run in completely different environments. In the first case the silk mats are completely immersed in PBS, which means that each fiber is surrounded by medium and thus the EGF contained in the fibers can easily diffuse through the silk material and dissolve in the medium. When the silk mats are put on top of the wound, only the fibers in direct contact with the wound are surrounded by a wet medium that could facilitate the diffusion of the EGF protein. That means only a small amount of EGF can diffuse out of the fibers. Furthermore, the ELISA measurements only give a measurement of the quantity of EGF released by the silk mats, other parameters need to be taken into account such as the fixation and sometime endocytosis of EGF by cells or the degradation of the protein by enzymes present in the culture medium or secreted by the cells.

3.4. Silk mats contacting the wound

We observed the structure of the silk mats before (Fig. 5a and b), after 72 h soaked in PBS at 37 °C (Fig. 5c and d), or after 72 h of contact with the wounds (Fig. 5e and f) using scanning electron microscopy. The overall structure of the mats was not perturbed and the random distribution of the fibers was still observed after 72 h of contact. The only visible difference was in the diameter of the fibers. After being in contact with the wound, the fibers presented areas of swelling. In these areas, the fiber's diameter was increased by more than one third (34.9%). Measurements of the average diameter of the fibers before (1.06 μm ± 0.03) and after the contact (1.43 μm ± 0.09) demonstrate a significant difference ($p < 0.01$) between the fibers. This observation can be explained by the absorption of liquid (culture medium) by the silk fibers diffusing through the gel in the wounded area in the absence of epithelium. Moreover, silk fibers soaked in PBS during 72 h present also an increase in their diameter (1.66 μm ± 0.03) but the size of the fibers is more regular as demonstrated by the calculation of the standard deviation used here as a quantification of the dispersion of the collected measurements (standard deviation of the fiber's diameter before contact and soaked: 0.16 and 0.18; standard deviation of the fiber's diameter after contact with the wound: 0.42). Our results link the change in fiber diameter with liquid uptake in this system. This absorption and the fact that the liquid is retained by the mats might also create a wet microenvironment over the wound improving the conditions needed for wound closure.

3.5. Wound reepithelialization

Twenty four or 48 h after wounding, tissues were stained by hematoxylin and eosin for morphological analysis. Wound closure was evaluated by measuring the distance between the wound edge and the tip of the epithelializing tongues as the percentage of wound closure. To assess the effect of the EGF on the wound reepithelialization, we compared wound closure of the control tissues (no silk mat placed in contact of the wound) after 24 (Fig. 6a) and 48 h (Fig. 6b) to either silk mat without EGF after 24 h (Fig. 6c) and after 48 h (Fig. 6d) or a silk mat containing EGF (Fig. 6e after 24 h and Fig. 6f after 48 h).

After 24 h we see only a small epithelial tongue beginning to cover the wound in the cases of nothing added on top of the wound and of silk mats (Fig. 6a and c). In contrast, the wound covered with the silk mats containing the growth factor EGF exhibit a longer epithelial tongue after 24 h (Fig. 6e). After 48 h, the control and the wound covered only with silk (Fig. 6b and d) show a similar response, the epithelial tongue is starting to cover the wound. In the case of the wound covered with the silk mat containing the EGF (Fig. 6f) after 48 h, the wound is

completely closed. During reepithelialization, no cells could be detected in direct contact with the mats.

In order to quantify wound closure, we calculated the percentage of reepithelialization after 24 (gray boxes) and 48 h (black boxes) (Fig. 7). The results show that after 24 h there is a slight difference between the control (wound closure of 2.6 ± 1.8%) and the silk mat without EGF (wound closure of 15.7 ± 9.1%). But in the case of the wound covered with the silk mats containing EGF, after 24 h, the wound closure is 84.8 ± 11.4%. After 48 h this difference was greater as wounds treated with EGF showed a 98.5 ± 1.5% closure, whereas control and the silk only exhibited wound closure of 8.9 ± 2.7% and of 26.3 ± 4.9%, respectively. Thus, the presence of EGF incorporated into the silk mat accelerated wound closure. We also tested a positive control by adding EGF (100 μg ml^{-1}) directly to the culture medium. Under these conditions, the closure percentage was 73.5 ± 10.2% after 24 h and completely close after 48 h, a closure pattern very similar to the one observed with the EGF-loaded silk mats.

4. Discussion

The aim of this study was to investigate the possibility of using wound dressings made of electrospun silk fibers functionalized with growth factors to help the closure of wounds by accelerating reepithelialization.

Our results first proved that EGF was incorporated into the silk fibers during the electrospinning process. More specifically, no demixing that could lead to a segregation of the growth factor EGF during the electrospinning occurs. Electrospinning is thus a good method to incorporate and release drugs or growth factors in silk fibers [25,26]. The incorporation and the release of protein in silk fibers has already be shown with the growth factor bone morphogenetic protein-2 (BMP-2), a known morphogen that induces osteogenesis in stem cells [13].

The ELISA test assay assesses the presence of EGF in the silk mats. Moreover, the acceleration of wound reepithelialization proves that the EGF present in the silk fibers is still active. This is an important point considering that the electrospinning is a stringent process that could potentially inactivate the protein. When EGF is released from the mat, we observed a burst effect. The reepithelialization of the wounds is a process starting immediately after the wounding. It needs to be fast as it is essential for the protection of the integrity if the body especially against microbes and water loss. This burst effect therefore is important for a rapid activation of the keratinocytes on the wound edge. In this material, it can be explained by the fact that the protein is not only inside the fiber but also at the surface. The first EGF molecules that diffuse into the medium are the ones on the surface of the silk fibers, which explain the rapid increase in EGF concentration in the medium and thus results in the burst seen on the release profile. The second part of the release profile shows a very smooth increase, where the EGF released is due to diffusion of the EGF molecules from inside the silk fibers to the fiber surface.

The wound closure by the epithelial cells is the first and most important step in wound healing. Our results demonstrate that the silk mats help reepithelialization of the wounded area by the keratinocytes tongue. In our experiments the silk mats act on two different levels, first by offering a physical protection to the wound and second by releasing EGF slowly and directly on the wound. The physical effect of the mats is demonstrated by the increase of wound closure rate observed after 24 or 48 h when nonfunctionalized mats are placed on the wound. As shown in the literature [27], this effect might be due to the wet environment created by the mat.

A major interest of electrospun silk mats for wound dressing is due to the fact that silk can be engineered to degrade with a slow rate while having a minimal negative impact on the surrounding tissues. In native fiber form, the highly crystalline fibers (>50% beta sheet content)

require months to years to fully degrade, due to a surface enzymatic hydrolysis reaction [28, 29]. But processing silk fibroin protein into biomaterial systems has included attempts to control the rate of degradation. In recent in vivo studies in mice, subcutaneous three-dimensional porous silk implants degraded in weeks (aqueous processing, low content of beta sheet) to over one year (solvent processing, high content of beta sheet) [29]. Moreover, silk fibers used as sutures (and FDA approved), are biocompatible and less immunogenic and inflammatory than collagens or polyesters such as polylactic acid [28,30,31]. Therefore, the slow degradation of silk mats, in contact with the wound, will not cause an inflammatory reaction and interfere with the healing process.

In this article, the topical effect of the molecule is demonstrated by the inclusion of EGF in the silk fibers, but bio-functionalization is not limited to one molecule or function and opens the way to the creation of multi-functionalized bioactive nanomaterials. Indeed, the coordinated effects of multiple growth factors in vivo are needed to direct wound closure in order to avoid undesirable responses such as keloid formation and scarring [32]. Moreover, a wound means that the integrity of the protective barrier has been ruptured and therefore increases the risk of infection. It may be possible to utilize multi-functionalized silk mats that incorporate antimicrobial molecules that could provide both anti-bacterial factors, as well as wound healing stimulants, to provide wound protection while stimulating wound closure.

To demonstrate the potential of such bioactive wound dressings, we used three-dimensional tissue-engineered models of human skin that have been developed because animal models have raised both safety and ethical concerns. For wound healing studies on human cells, prior existing models used only a single cell monolayer such as fibroblasts, epithelial (mostly keratinocytes), or endothelial cells [33]. These models allow the study of cellular behavior and different factors that are involved in cellular processes such as migration, proliferation, and protein synthesis. Some extra-cellular matrix [34,35] and growth factors [36,37] have been added to these models, but these 2D models have limitations and do not accurately model the complex wound healing process. Therefore, it was essential to test the biofunctionalized silk mats on human three-dimensional tissues which display the architectural, morphological, and biochemical features seen in human skin to further understand the effect this new biomaterial in their appropriate tissue context. Furthermore, we have adapted human skin-equivalents to study reepithelialization and wound response in a tissue that mimics the chronology of events that occur following wounding of human skin [38–41]. These three-dimensional tissue-engineered models of wounded human skin have been used to test other types of biomaterials such as hydrogels [41]. Altogether, human skin-equivalents are a recognized alternative to animal testing for scientific laboratories as well as for industry to study not only wound healing [41,42], but also skin corrosion [43], skin irritation [44], or toxicity [45] as a preclinical step. Our results, obtained on human skin-equivalents, demonstrate the potential of such biologically functionalized dressing to accelerate wound reepithelialization and its possible use as a clinical therapeutic application.

5. Conclusions

Our results demonstrate that biofunctionalized silk mats containing active molecules represent an extremely promising method to achieve bioactive wound dressings. Such topical modes of actions are of great interest for biomedical applications in general, and especially for wound healing, as they represent a new type of material to fight the growing problem of chronic non-healing wounds.

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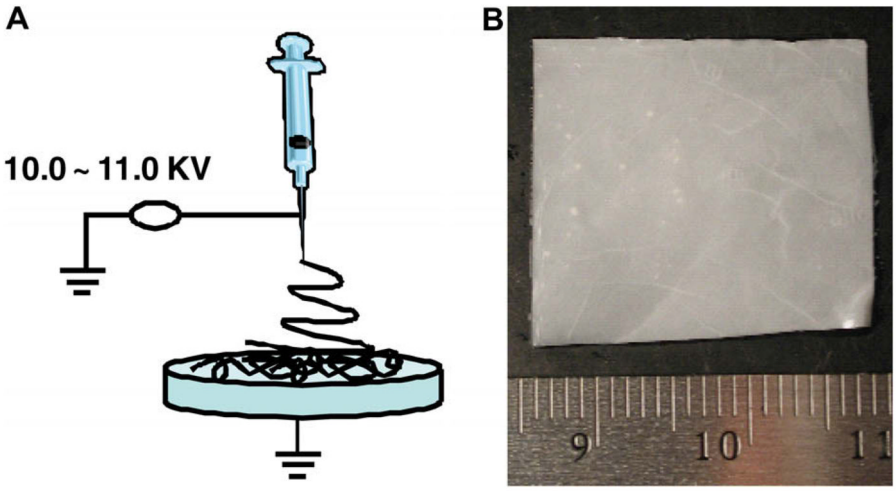

Fig. 1.

(A) Electrospinning apparatus for silk mat preparation. The fibroin solution is mixed with the growth factor in the syringe before electrospinning the silk. (B) Methanol treated EGF-incorporated silk mat. The mat appears like a small piece of tissue that can be cut to fit the wound.

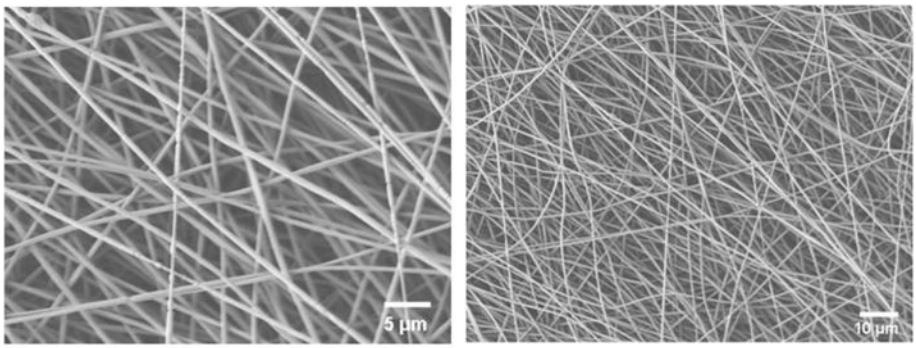

Fig. 2.
SEM pictures of the electrospun silk mats. The mats consist of randomly distributed silk fibers of about 1 μm in diameter

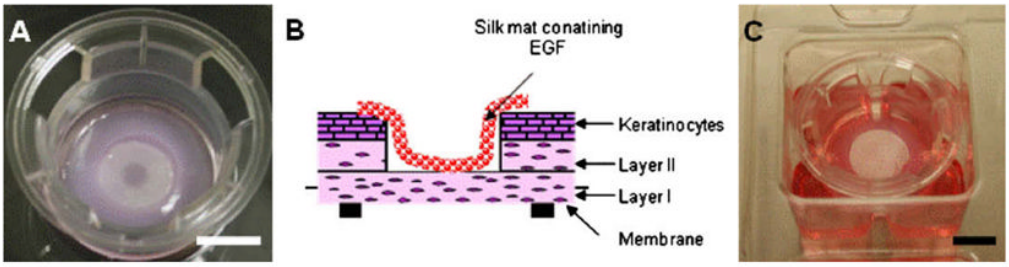

Fig. 3.

Construction of composite organotypic co-culture wound healing model. A wound is formed by punching a hole through the epithelium and collagen matrix. (A) Picture of the wound after the transfer of the punched human skin-equivalent onto a second collagen matrix that has undergone contraction (layer I), the resultant composite co-culture consists of two layers of contracted matrix and one layer of epithelium. (B and C) Schematic and picture of the wound with the silk mat, reepithelialization occurs as wounded keratinocytes migrate under the silk mats onto the collagen in layer I. The percentages of wound closure are a measure of the distance between the edges and between the epithelial tongues to obtain the percentage of wound closure. Scale bar (A) and (C): 10 mm.

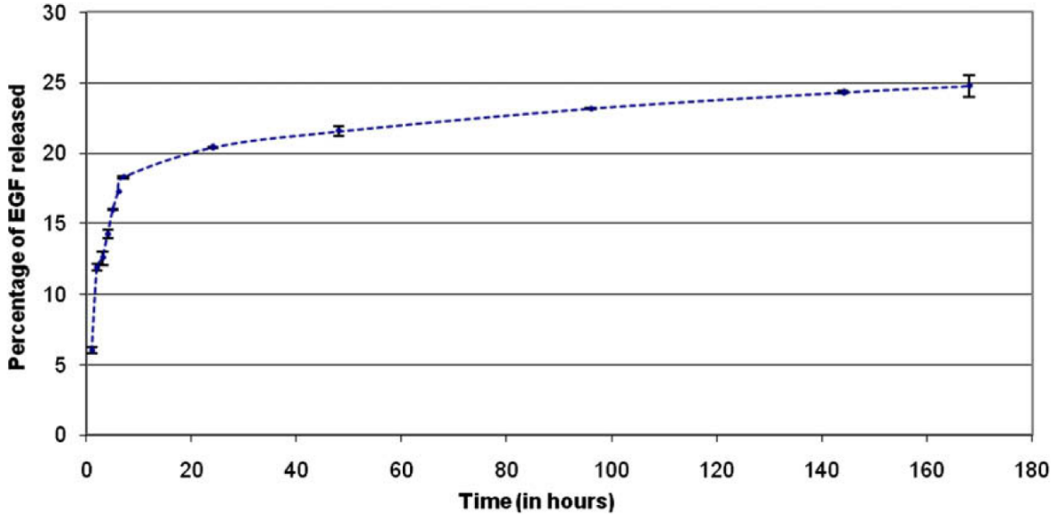

Fig. 4.
In vitro release profile of EGF from the electrospun silk mat at 37 ± 0.5 °C (*n* = 6) over 7 days.

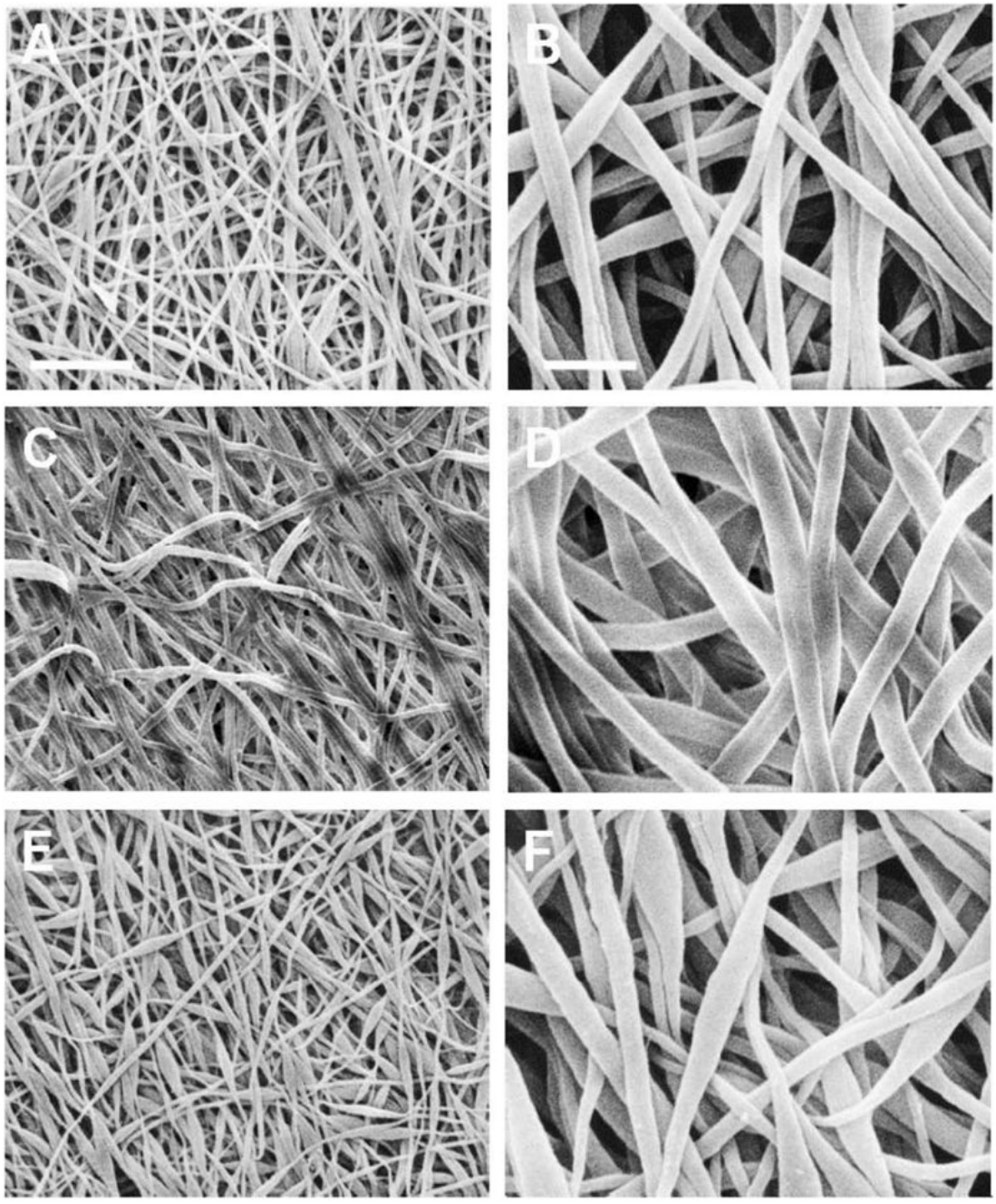

Fig. 5.
SEM pictures of the electrospun silk mats before contact on the wound (A and B), after 72 h soaked in PBS at 37 °C (C and D) and after contact on the wound for 72 h (E and F). Note the difference observed on the diameter of the fibers and the irregularities in the diameter of the fibers that contacted the wound. Bars: (A, C, and E): 20 μm; (b, d, and f): 5 μm

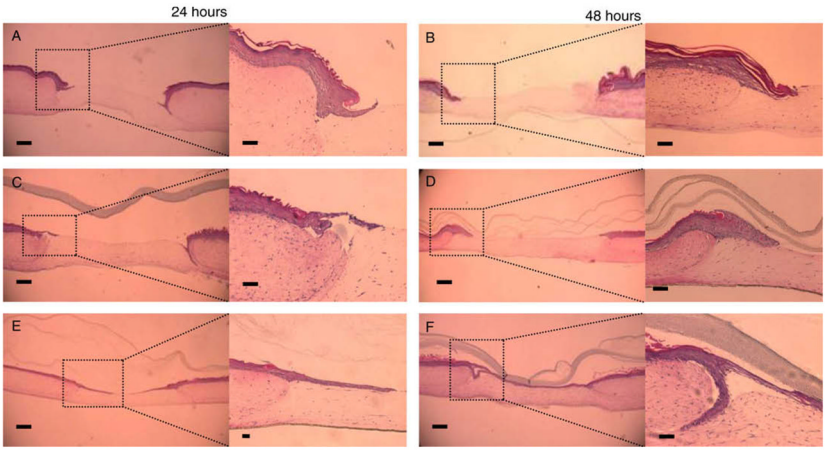

Fig. 6.

Hematoxylin and Eosin staining of the wound after 24 (left column) and 48 h (right column). (A) and (B) are controls, without the silk dressing on top of the wound. (C) and (D) are the samples on which we applied a silk mat without EGF. (E) and (F) are the samples on which a silk mat containing the EGF has been applied. Lower magnification: scale bar equals 100 μm, higher magnification: scale bar equals 300 μm.

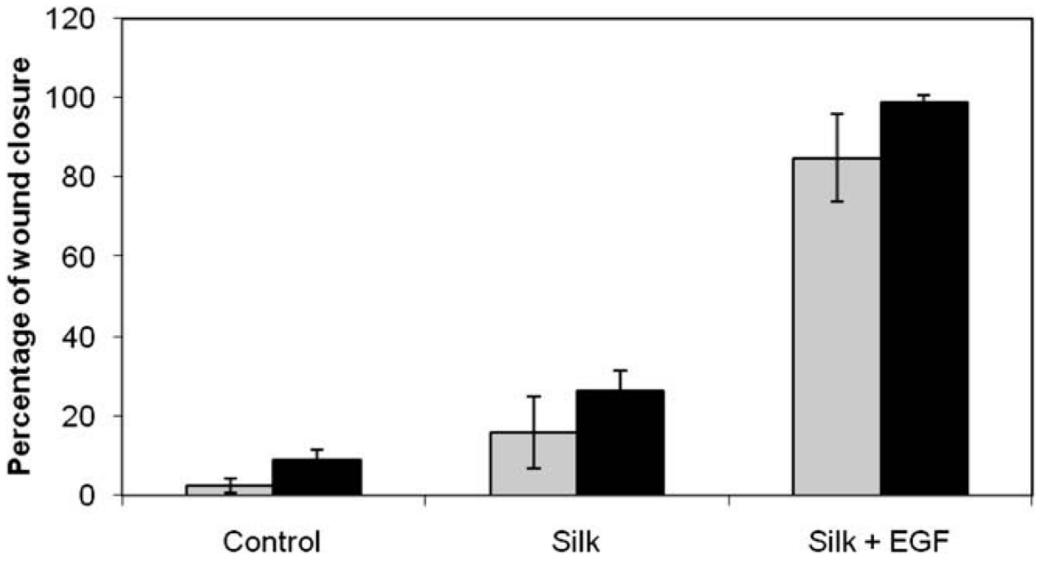

Fig. 7.

Percentage of wound closure after 24 (gray) and 48 h (black). Data points are the average of $n = 7$ and error bars represent ± SD.