含銀活性碳纖維敷料應用於促進傷口癒合之應用探討

Evaluation of Silver-Containing Activated Carbon Fiber for Wound Healing Study: *in vitro* and *in vivo*

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中文摘要

發展可一抗菌與吸收組織液的醫用敷料，是目前臨床中傷口癒合的重要課題，例如：美國 Argentum Medical 公司的 Silverlon 產品，其醫用敷料性能皆較其餘敷料效果優異，更受到醫護人員與病患歡迎。根據 Espicom Business Intelligenc 市場調查顯示，傷口敷料市場預估到 2012 年可達 125 億美金，具高效能性傷口敷材更具是高經濟價值，可惜的是，在我國傷口醫療的含銀抗菌與吸收組織液敷料市場中，皆尚未可自行研發出醫用敷材產品。科云生醫科技股份有限公司研發載銀活性碳纖維高性能傷口敷料，獲得多國專利和通過 CE、ISO13485 與 GMP 認證。本研究拿公司含銀活性碳纖維覆蓋在老鼠傷口組織，發現載低銀濃度活性碳纖維技術可有效抑制臨床菌株抑菌能力和降低對纖維母細胞的毒性。同時在動物傷口癒合狀況得知，科云生醫科技股份有限公司研發載銀活性碳纖維傷口敷料傷口癒合效果優越美國 Argentum Medical 公司的 Silverlon 並有效降低傷口發炎性質和提升皮膚組織重建效果，更具有臨床可應用性。

關鍵詞：敷料、載銀活性碳纖維、組織重建

Abstract

Silver has antiseptic properties, anti-inflammatory properties, and is a broad-spectrum antibiotic for multidrug-resistant strains of bacteria. The commercially available product Silverlon® is a silver-plated three-dimensional polyamide fabric with a high silver concentration of 546 mg/100 cm^2. Thus, fibroblast cell growth is affected when exposed to the Silverlon® treated cell medium. Our study produced an Ag-activated carbon fiber wound dressing that incorporated various silver concentrations (in cooperation of Bio-Medical Carbon Technology Co.) to examine antimicrobial properties and determine fibroblast cell viability upon exposure to the silver impregnated dressing material as compared to other commercially available products such as calcium alginate dressing, Sorbalgon® and silver-polyamide fabric dressing, Silverlon®. The silver impregnated activated carbon fiber dressing induced less damage to fibroblast cells compared to the effect produced by Silverlon® and exhibited similar antibacterial abilities in vitro. *In vivo* analysis showed that various silver concentrations impregnated activated carbon fiber dressings promoted tissue reconstruction for wound with *Pseudomonas aeruginosa* infected wounds.

Keywords: Wound Dressing, Ag-Activated Carbon Fiber, Tissue Reconstruction

Introduction

An ideal wound dressing needs to efficiently absorb tissue fluids, prevent bacterial infection, and reduce the severity of inflammation. There are many wound dressings available that absorb wound exudates, for example, Sorbalgon®, which is made of calcium alginate. Another challenge facing caregivers is the growing incidence of infection by antibiotic−resistant bacterial strains in combat trauma wounds [1,2]. Gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) and Gram-positive organisms (*Staphylococcus aureus*) are common environmental contaminants of burn wounds and other traumas [3]. However, organisms that become resistant to these antibiotics are among the most disconcerting multidrug-resistant pathogens [4].

Silver (Ag) has antiseptic and anti-inflammatory properties as well as a broad-spectrum antibiotic for multidrug-resistant strains of bacteria [5]. Ag has a potent antimicrobial effect that destroys microorganisms immediately by blocking cellular respiration and disrupting the function of bacterial cell membranes, or by binding and denaturing bacterial deoxyribonucleic acid or ribonucleic acid to inhibit cell replication [6]. Silverlon® (Argentum Medical, Lakemont, GA, USA) is a silver-plated three-dimensional polyamide fabric that is fabricated using a proprietary autocatalytic electroless chemical (reduction-oxidation) plating technology. The material has been used as a topical agent in medicine. Silverlon® has a high silver concentration of 546 mg/100 cm^2 in its polyamide fabric; fibroblast cell viability tests showed that cell growth was affected when exposed to Silverlon® treated cell medium.

In our study, Bio- Medical Carbon Technology Co. had used impregnate method of fabricating polyacrylonitrile- based activated carbon fiber (ACF) to support the silver component and produce a suitable wound dressing with good antimicrobial properties that also reduces cell cytotoxic affection. Our objectives were to produce ACF wound dressings that incorporate various Ag concentrations with the cooperation of Bio-Medical Carbon Technology Co., to examine its physicochemical characteristics. Additionally, we preformed *in vivo* experiments in rat models to compare wound healing with the prepared Ag-containing ACF wound dressing versus commercially available products (calcium alginate dressing, Sorbalgon®; silver-polyamide fabric dressing, Silverlon®). We observed wound healing in injured rats infected with *P. aeruginosa* and treated them with activated carbon fiber dressing containing various silver concentrations [25 mg/100 cm^2 (Ag-25/ACF) or 50 mg/100 cm^2 (Ag-50/ACF)].

Materials and Methods

Fabrication of Ag-containing ACF wound dressing

The ACF was produced from raw oxidized polyacrylonitrile fiber cloths. The precursor of oxidized polyacrylonitrile fiber was treated with phosphoric acid and then was co-activated using steam at a flow rate of 0.2 mL/min for 10 min. The ACF samples were impregnated with silver acetate solution in vacuo for 180 min, then heated at 350°C for 120 min under nitrogen. The silvered sample was then broken into fine grains and deposited on the ACF in preparation for further study. The morphologies of the prepared Ag-containing ACF samples were examined using a scanning electron microscope (SEM). All of the test samples were mounted on metal grids using double-sided carbon adhesive tape, and the SEM was used at an accelerating voltage of 8 kV.

Animal study

Male Sprague–Dawley (SD) rats were anesthetized with an intraperitoneal injection of ketamine (90 mg/kg) with xylazine (10 mg/kg). The dorsal area skin was shaved, application fields were outlined with a marker pen before skin excision, and the surgical area was disinfected with 70% ethanol. On the back of each rat, a full-thickness wound with dimensions of 1×1 cm^2 was created on each side of the spine by dermoepidermic excision. Group 1 was the Sorbalgon® dressing-treated group; group 2 was the Silverlon® dressing-treated group; and group 3 was the prepared Ag-25/ACF dressing-treated group. Each dressing with dimensions of 2×2 cm^2 was covered with a sterile compress secured by a hypoallergenic elastic adhesive bandage. Animals were caged individually following identification. The dressings were removed on days 3, 6, 9, 12, and 15 post-surgery, and the wounds were examined and photographed to measure wound size reduction. Meanwhile, following model of infected wounds, the wounded rats each received 50 μL of PBS containing 5×10^7 colony-forming units of mid-log culture *P. aeruginosa* on the wound surface. Rats were dressed with Ag-25/ACF or Ag-50/ACF dressings 30 min after infection. Each dressing was covered with a sterile compress secured by a hypoallergenic elastic adhesive bandage. Animals were caged individually following identification. The dressings were removed every third post-surgery day; the wounds were photographed and examined to determine wound size reduction. Wound size measurements taken during surgery and biopsy were used to calculate percentage size reduction of wounds. Wound area was measured from photographs using the Image-Pro Plus (Media Cybernetics, Silver Spring, MD, USA) following calibration. Histologic analysis at different healing times was carried out using light microscopy. Briefly, biopsies were fixed in buffered paraformaldehyde and embedded in paraffin wax. Sections of approximately 5 μm were stained with Hematoxylin and eosin, and Masson’s trichrome. The stained sections of each test sample were then examined under a light microscope, for analysis of tissue inflammatory reaction and regeneration.

Results and Discussion

Micrographs of Ag-containing ACF

The prepared ACFs, impregnated with silver acetate solution, were heated in an oven at 350°C for 120 min under nitrogen to form fine grains. The original dimension of the prepared ACF wound dressing containing silver is with dimensions of 10×10 cm^2. Figure 1 shows the SEM micrographs of Ag-25/ACF, Ag-50/ACF, and Ag-plated polyamide fabric (Silverlon®). The micrographs, show that the distribution of Ag particles plated on ACF has a uniform distribution, and in the pictures, the quantity of Ag particles plated on Ag-50/ACF is much greater than that seen for Ag-25/ACF (black arrows). Silverlon® is an Ag-polyamide fabric dressing. During manufacture, silver nitrate is used to incorporate silver metal into the fabric to a silver concentration of 546 mg/100 cm^2 using an autocatalytic electroless chemical plating technology. Figure 1 shows the SEM micrograph of Silverlon® shows a greater density of silver particles than that present in either the Ag-25/ACF or Ag-50/ACF sample micrographs.

In vivo wound healing

We observed clear differences in wound closure between the various different wound dressing samples at 3, 6, 9, 12, and 15 days (Figure 2). It was found that the wound closure efficiency for Ag-containing dressings, including Silverlon® and Ag-25/ACF were better than Sorbalgon®. Porous ACF has high adsorption, which is beneficial for medical applications, and it is both biocompatible and bioactive, making the material ideal for wound dressing applications or for use as a three-dimensional cell scaffold. Ag-ACF air permeability was 108.62 ± 14.83 $cm^3/cm^2/s$ was greater than that of Silverlon® at 29.21 ± 5.76 $cm^3/cm^2/s$. For faster healing, wounds need to be well oxygenated depending on the air permeability of the dressing. The reduction rate for the wound defect area was calculated by measuring the wound area at different times (Figure 3). Figure 3 shows, after three days, the wound closure for the various test dressings were 24.68 ± 5.07% for Sorbalgon®, 35.81 ± 3.59% for Silverlon®, and 45.11 ± 4.68% for Ag-25/ACF. After six days, wound closure using Ag-25/ACF was 69.73 ± 3.64% was better than Silverlon® at 60.21 ± 4.83% and Sorbalgon®, 48.64 ± 3.85%. These differences were most apparent at day 9 and 12, with wounds treated with the Ag-25/ACF wound dressing showing 90.75 ± 7.06% and 93.36 ± 7.71% closure, and compared to 81.13 ± 6.42% and 89.93 ± 2.51% for Silverlon® and 73.21 ± 2.96% and 83.31 ± 6.34% for Sorbalgon®.

Histological evaluation of healing impaired wounds

Figure 4(a) shows histological results for Ag-25/ACF, and the Sorbalgon® control, when applied to a dorsal skin wound on Sprague-Dawley rats. On day three, the Sorbalgon® treated wound showed more loose connective tissue and more severe inflammation than the Ag-25/ACF treated wound did. Additionally, the Sorbalgon® treated wound presented a large number of inflammatory cells that had penetrated the intramuscular tissue beneath the wound (red arrows). On day six, wounds dressed with the composite Ag-25/ACF exhibited some neo-capillary formation (red circles) and the degree of inflammation was less than that on day three [Figure 4(a)]. Conversely, wounds treated with Sorbalgon® still presented considerable inflammation on day six, particularly at the wound periphery (red arrows). Figure 4b shows Hematoxylin and eosin and Masson's trichrome staining of rat tissue samples after nine days of treatment with Ag-25/ACF. The histological examination was carried out at ×400 magnification under a light microscope, the tissue inflammatory reaction and regeneration were analyzed. Neo-capillary formation (red circles) in the Ag-25/ACF treated rats was confirmed by Hematoxylin and eosin staining. We identified neo-connective-tissue fibrils (blue arrows) in the same animals as neocollagen regenerated by the rat hosts upon staining with Masson's trichrome. These findings show that applying Ag-25/ACF dressings promotes tissue reconstruction and demonstrates the potential of the Ag-25/ACF as a skin substitute.

Effect of Ag-25/ACF and Ag-50/ACF on healing of infection wounds

Infected wounds heal more slowly and less effectively than non-infected wounds do; a large part of the wound care market is concerned with the antibacterial effects of various topical preparations and wound dressings. Activated carbon fiber offers high adsorption and catalytic activity. These properties are beneficial for medical applications, water treatments, and air treatments for their ability to remove organic and inorganic pollutants. The adsorption properties of activated carbon have been demonstrated to clear fluids of bacterial endo- and exotoxins. *P. aeruginosa*: a species of gram-negative, aerobic, rod-shaped bacteria commonly isolated from clinical specimens is a major agent of nosocomial infection. It was reported that Ag antimicrobial mechanisms include: (*i*) Ag^+ ions bind to sulfhydryl groups, which leads to protein denaturation by reduction of disulfide bonds; (*ii*) Ag^+ complex with electron donor groups containing sulfur, oxygen or nitrogen that are normally present as thiol or phosphate groups in amino acids and nucleic acids. Bio-Medical Carbon Technology Co. used a vacuum method to prepare ACFs containing Ag for use in bactericidal applications. ACF dressings were prepared with silver concentrations of 25 mg/100 cm^2 (Ag-25/ACF) and 50 mg/100 cm^2 (Ag-50/ACF). The dressings were applied to infected excisional wounds in infected SD rats. Figures 5 and 6 show the time courses of excisional wound healing in *P. aeruginosa* infected wounds. The figures show that the wound closure efficiency of the Ag-50/ACF was greater than that of the Ag-25/ACF dressing for all time intervals (Figure 5). Figure 6 shows that the average wound area seen for Ag-25/ACF treated rats after three days was 63.89 ± 7.09 mm^2, after six days the area had reduced to 37.27 ± 3.16 mm^2, after nine days it was 14.25 ± 2.51 mm^2, at 12 days 8.64 ± 1.44 mm^2, and by 15 days, it was just 3.61 ± 0.28 mm^2. Average wound areas for Ag-50/ACF treated rats were 51.21 ± 4.08 mm^2 at 3 days, 27.82 ± 3.84 mm^2 at 6 days, 8.89 ± 1.83 mm^2 at 9 days, 4.07 ± 1.11 mm^2 at 12 days, and 2.75 ± 0.14 mm^2 at 15 days. Specifically, Ag-50/ACF dressings showed a significantly increased rate of wound healing with *P. aeruginosa* infected wounds compared to with Ag-25/ACF dressings.

Conclusions

The produced activated carbon fiber wound dressings that incorporate various silver concentrations with the cooperation of Bio-Medical Carbon Technology Co. The SEM micrograph shows that the distribution of Ag particles plated on ACF has a uniform distribution. An *in vivo* analysis showed that Ag-25/ACF dressings promoted tissue reconstruction and neocollagen regeneration. Additionally, Ag-50/ACF dressings showed a significantly increased rate for the wound healing in rats with *P. aeruginosa* infected wounds.

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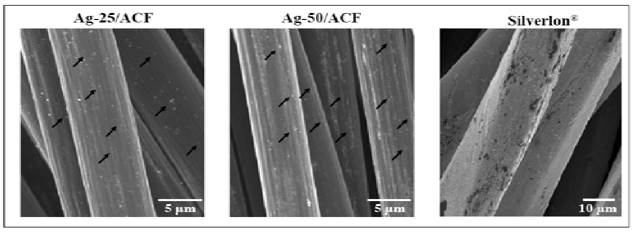

Figure 1. Scanning electron microscope micrographs of the wound dressings.

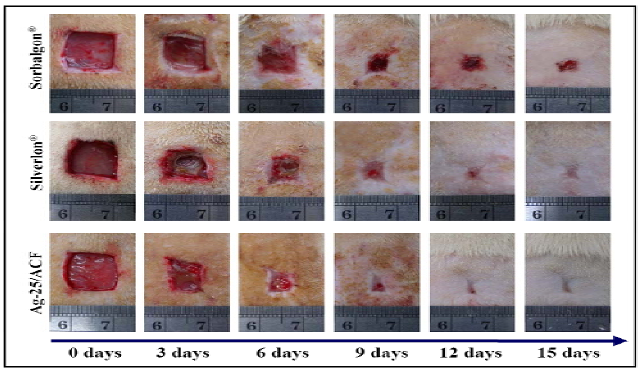

Figure 2. Representative photographs of the wound on days taken postoperatively following excision.

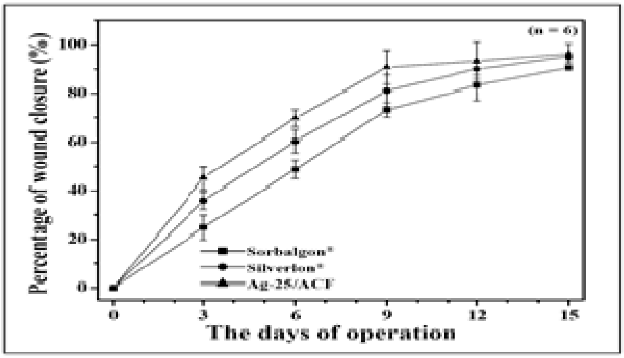

Figure 3. The percentage of wound closure following distinct time intervals and data points are the average of n = 6 and error bars represent ± SD.

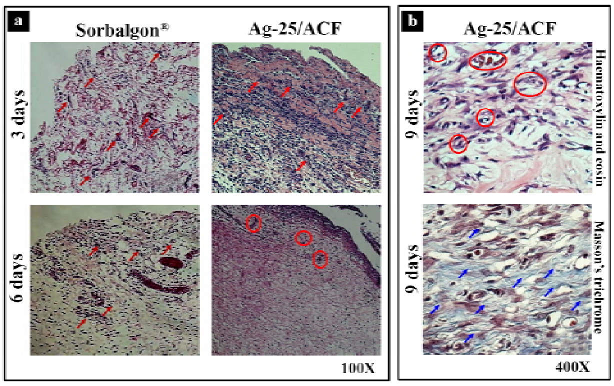

Figure 4. (a) The wounds treated with Sorbalgon® and Ag-25/ACF with staining Haematoxylin–eosin. **(b)** The wounds treated with Ag-25/ACF with Haematoxylin–eosin and Masson's trichrome.

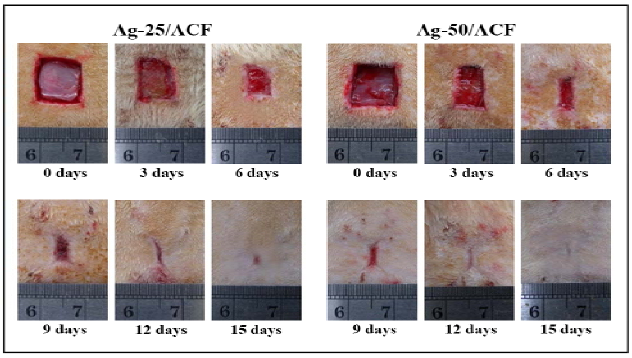

Figure 5. Representative photographs of the *Pseudomonas aeruginosa* infected wounds, taken postoperatively following skin excision.

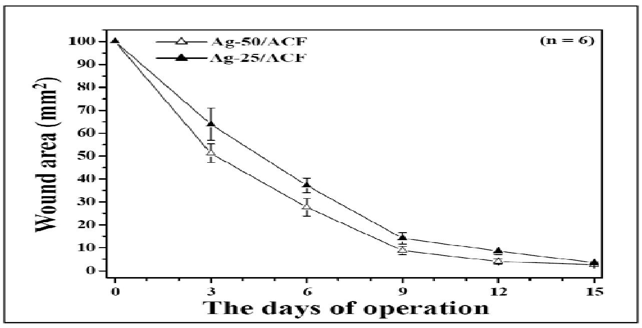

Figure 6. Wound areas of the *Pseudomonas aeruginosa* infected wounds following distinct time intervals and data points are the average of n = 6.