傷口治療及藥物載體之研發超透明蠶絲醫材應用於

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2 張晃猷
3 楊啟裕

一、前言

行政院農業委員會苗栗區農業改良場（簡稱苗改場）與國立清華大學及財團法人農業科技研究院共同合作，完成超透明蠶絲蛋白敷料的技術開發及功效評估。此研發成果不僅成為2020年亞洲生技大展亮點報導（圖1），製程技術也在今年取得中華民國發明專利，成果受到國際期刊之刊登及矚目，近日已完成醫療器材查驗登記中的臨床前測試文件，相關醫材產品上市指日可待。

絲（Silk）為昆蟲生產的纖維性蛋白質，蠶絲是最早開發也是現今最大量為人類使用的

圖1. 行政院農業委員會陳駿季副主任委員等蒞臨2020年亞洲生技大展瞭解亮點主題——高透明蠶絲蛋白敷料研發進展。

註1：行政院農業委員會苗栗區農業改良場。
註2：國立清華大學分子醫學研究所。
註3：財團法人農業科技研究院。

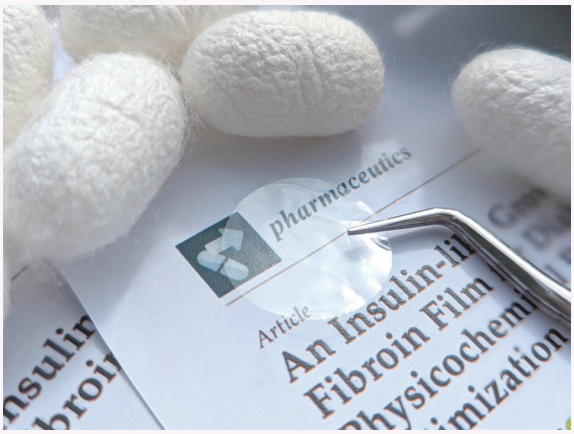
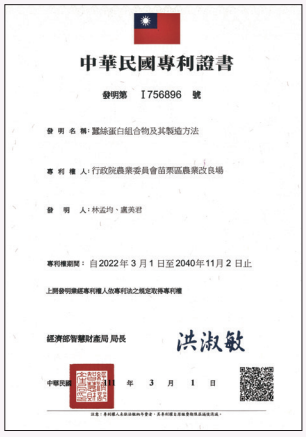


圖2. 蠶絲蛋白透過技術加值成為超透明的生物醫材。


中華民國專利證書

發明第 I756896 號

發明名稱：蠶絲蛋白組合物及其製造方法

專利權人：行政院農業委員會苗栗區農業改良場

發明人：林孟均、盧美君

專利權期間：自2022年3月1日至2040年11月2日止

上開發明業經專利權人依專利法之規定取得專利權

經濟部智慧財產局 局長 洪淑敏

中華民國 111 年 3 月 1 日
圖3. 超透明蠶絲蛋白敷料之發明專利。

昆蟲絲蛋白，蠶絲由家蠶（*Bombyx mori* L.）體內的絹絲腺所生合成，占家蠶體重的40%～50%，依結構可粗分為絲膠蛋白（Sericin）及絲質蛋白（Fibroin），其中的絲質蛋白因具有高穩定性、高機械力及生物親和性等人造材料無法取代的優勢，近年來受到材料科學、生物醫學、美容保健，甚至半導體產業高度重視。在生醫的範疇中，蠶絲蛋白被視為能加速組織修復之新興材料，傷口治療是提升醫療品質的關鍵技術，市場蓬勃發展。臺灣及全球的敷料年複合成長率高達9.2%及6.2%，預估2024年總產值高達42億美元，其中又以治療難癒合的糖尿病慢性潰瘍市場需求量最高。但盤點市售敷料產品，缺乏具有低敏性、專一性治療及長效治療等功能之糖尿病專用敷材。有鑑於此，苗改場與國立清華大學及財團法人農業科技研究院共同合作，以絲質蛋白為材料開發糖尿病傷口專用高階敷料，期能成功跨域生醫新產業，拓展蠶桑產業新價值。

二、蠶絲蛋白化身超透明的生物醫材

傳統加工處理技術局限了蠶絲原料的效能，本研究透過製程的進化，大幅移除易致敏原——絲膠蛋白，並利用後續提純及灌注技術優化，不僅可降低產製成本，更進一步提高了絲質蛋白的純度40%以上，此技術增加敷料蛋白質結構中的β摺板（Beta Sheet），提升了原料的穩定度，最終完成獨步全球的超透明蠶絲薄膜型敷料（圖2）：經細胞試驗證實此敷料可促進刻痕中細胞的爬行速度達30%以上，並可在蠶絲薄膜上增殖出大量細胞，後續可運用在再生醫材及人造器官。研發衍生之「蠶絲蛋白組合物及其製造方法」已取得中華民國發明專利（發明第I756896號）（圖3）。

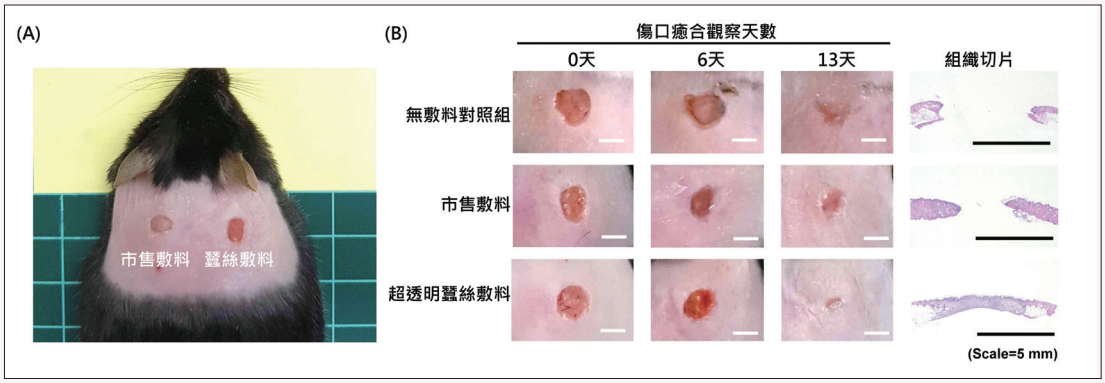


圖 4. 超透明蠶絲蛋白敷料可促進糖尿病鼠傷口 (A)；微血管及癒傷組織再生 (B) 加速傷口癒合 (市售敷料係指水膠體人工皮)。

三、超透明蠶絲蛋白敷料運用於糖尿病動物傷口治療

相較於市售常見的水膠體人工皮（Hydrocolloid），超透明蠶絲敷料在動物傷口確效性試驗中，可促進糖尿病鼠傷口微血管再生及癒傷組織形成，進而加速傷口修復，整體癒合率提升 20% 以上（圖 4），此外，為了符合我國第二級醫療器材查驗登記要件，110 年起委請農科院協同進行糖尿病豬的臨床前測試，結果顯示此敷料無皮膚刺激性，皮內注射呈輕度至無刺激性，顯示其作為敷料之無過敏性優勢。在豬隻傷口癒合功效評估試驗中，相較於牛源性膠原蛋白，蠶絲敷料有較佳的消炎反應，能加速傷口上皮增殖，促進膠原蛋白增生等傷口修復。綜合上述，顯示此敷料較市售主流敷料（人工皮及膠原蛋白）更具有市場競爭力。

四、蠶絲蛋白作爲藥劑緩釋載體

除了促進傷口癒合之外，我們也發現蠶絲蛋白敷料具有藥物承載及釋放的功效。經藥物動力分析，超透明蠶絲敷料結合類胰島素生長因子（IGF-1），IGF-1 的釋放速率會遵循零級釋放的動力學（Zero-order Kinetics）。相較於游離型生長因子，經敷料釋放可將 IGF-1 活性自 15 分鐘延長至 30 天以上，藉此可用來調控藥物在體內的濃度恆定，減少給藥頻率和副作用產生。此外，相較於口服用藥，利用敷料直接施用在患部上也可達到精準投藥的效果，降低傷口治療所需成本。相關成果已陸續發表於國際期刊《藥劑學》（*Pharmaceutics*）（IF: 6.321 藥學領域排名 10.33%）及《國際分子科學雜誌》（*International Journal of Molecular Sciences*）（IF: 5.924 生化與分生領域排名 22.54%）

（圖5），未來也會朝著技術移轉及產品上市方向努力，持續推動農業跨域生醫的新創事業育成。

五、結語

隨著國際市場及產業結構的改變，傳統的栽桑養蠶逐漸式微，所幸隨著各領域創新技術的崛起，賦予了蠶絲全新的使命，以絲質蛋白原料為例，全球需求量的年複合成長率就高達6.6%，預估至2027年全球絲質蛋白總需求產值上看1.4億美元，市場潛力不容小覷。未來蠶絲敷料量產技術將進一步與其他醫療材料產業接軌，以現有蠶絲蛋白創新製程，延伸應用於其他醫療產品，並進行產業化推廣應用。相信在不久的將來，相關產品就可投入市場造福群眾，讓臺灣農業跨域生醫產業的軟實力驚豔全世界。

International Journal of Molecular Sciences

Article

Sustained Release of Insulin-Like Growth Factor-1 from *Bombyx mori* L. Silk Fibroin Delivery for Diabetic Wound Therapy

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Abstract: The goals of this study are to develop a high purity patented silk fibroin (SF) film and test its suitability to be used as a slow-release delivery for insulin-like growth factor-1 (IGF-1). The release rate of the SF film delivering IGF-1 followed zero-order kinetics as determined via the Ritger and Peppas equation. The release rate constant was identified as 0.11, 0.23, and 0.09% h^{-1} at 37 °C for SF films loaded with 0.65, 6.5, and 65 pmol IGF-1, respectively. More importantly, the IGF-1 activity was preserved for more than 30 days when complexed with the SF film. We show that the IGF-1-loaded SF films significantly accelerated wound healing in vitro (BALB/3T3) and in vivo (diabetic mice), compared with wounds treated with free IGF-1 and an IGF-1-loaded hydrocolloid dressing. This was evidenced by a six-fold increase in the granulation tissue area in the IGF-1-loaded SF film treatment group compared to that of the PBS control group. Western blotting analysis also demonstrated that IGF-1 receptor (IGF1R) phosphorylation in diabetic wounds increased more significantly in the IGF-1-loaded SF films group than in other experimental groups. Our results suggest that IGF-1 sustained release from SF films promotes wound healing through continuously activating the IGF1R pathway, leading to the enhancement of both wound re-epithelialization and granulation tissue formation in diabetic mice. Collectively, these data indicate that SF films have considerable potential to be used as a wound dressing material for long-term IGF-1 delivery for diabetic wound therapy.

Keywords: *Bombyx mori* L.; insulin-like growth factor-1 (IGF-1); silk fibroin; wound healing; diabetes

1. Introduction

The skin is a large organ that plays an essential role in maintaining homeostasis and also protecting the body from microorganism invasion. Severe skin damage can result in infections, loss of body temperature and fluid balance, and often death [1,2]. Chronic wounds accompanied by inflammation and delayed wound repair commonly cause the disruption of the period of wound healing [3], which consumes great resources of healthcare. More than 6.5 million chronic skin ulcer cases primarily caused by blood pressure, venous stasis, or diabetes mellitus are reported annually in the United States, with treatment costs estimated to exceed USD 20 billion [4,5]. Although a number of products have been developed to improve chronic wound healing, no efficient therapy has been applied so far [6].

Growth factors have been extensively studied for their efficacy in accelerating chronic wound repair [7,8], with the majority of research focusing on epidermal growth factor, fibroblast growth factors, platelet-derived growth factor, vascular endothelial growth factor, and insulin-like growth factors. Many studies have revealed the potential of these growth factors to improve wound closure, granulation tissue formation, and angiogenesis [9]. A gel formulation of recombinant platelet-derived growth factor-BB (REGRANEX®) was approved for treating diabetic ulcers in the United States and Europe [10]. Despite their

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pharmaceutics

Article

An Insulin-like Growth Factor-1 Conjugated *Bombyx mori* Silk Fibroin Film for Diabetic Wound Healing: Fabrication, Physicochemical Property Characterization, and Dosage Optimization In Vitro and In Vivo

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Abstract: This study aimed to develop a silk fibroin (SF)-film for the treatment of chronic diabetic wounds. Silk fibroin was purified through a newly developed heating degumming (HD) process and casted on a hydrophobic surface to form SF-films. The process allowed the fabricated film to achieve a 42% increase in transparency and a 35% higher proliferation rate for BALB/3T3 fibroblasts compared to that obtained by conventional alkaline degumming treatment. Fourier transform infrared analysis demonstrated that secondary structure was retained in both HD- and alkaline degumming-derived SF preparations, although the crystallinity of beta-sheet in SF-films after the HD processing was slightly increased. This study also investigated whether conjugating insulin-like growth factor-1 (IGF-1) would promote diabetic wound healing and what the optimal dosage is. Using BALB/3T3 cells grown in hyperglycemic medium as a model, it was demonstrated that the optimal IGF-1 dosage to promote the cell growth was approximately 0.65 pmol. Further analysis of wound healing in a diabetic mouse model indicated that SF-film loaded with 3.25 pmol of IGF-1 showed significantly superior wound closure, a 13% increase at the 12th day after treatment relative to treatment with 65 pmol of free IGF-1. Improvement in diabetic wound healing was exerted synergistically by SF-film and IGF-1, as reflected by parameters including levels of re-epithelialization, epithelial tissue area, and angiogenesis. Finally, IGF-1 increased the epithelial tissue area and micro-vessel formation in a dose-dependent manner in a low dosage range (3.25 pmol) when loaded to SF-films. Together, these results strongly suggest that SF-film produced using HD and loaded with a low dosage of IGF-1 is a promising dressing for diabetic wound therapy.

Keywords: silkworm; fibroin; insulin-like growth factor-1; diabetic wound; dosage-dependent effect

1. Introduction

Diabetes mellitus is one of the most prevalent non-communicable diseases, affecting 422 million adults in 2014 and causing about 1.5 million deaths in 2012 [1]. Approximately 15% of diabetic patients suffer from chronic wounds or foot ulcers that frequently lead to limb amputation or even death [2,3]. In 2010, approximately 75,000 amputations were performed on diabetic patients with non-healing wounds in the United States [4]. Currently, the standard practices in diabetic wound management include surgical debridement, systematical glycemic control, wound off-loading, and dressings [5,6]. Although significant efforts have been exerted to improve diabetic wound healing, no satisfactory therapy has been developed thus far [7]. Therefore, more research on novel interventions to improve healing of diabetes-associated chronic wounds is urgently required.

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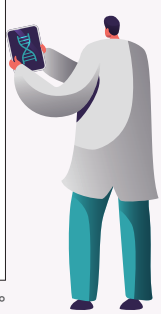

圖5. 超透明蠶絲敷料相關傷口治療及藥物動力評估已刊登於國際期刊《藥劑學》及《國際分子科學雜誌》。