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Electrospun polyurethane-dextran nanofiber mats loaded with Estradiol for post-menopausal wound dressing

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ABSTRACT

Post-menopausal wound care management is a substantial burden on health services, since there are an increased number of elderly populations linked with age-related delayed wound healing. The controlled estrogen replacement can accelerate healing of acute cutaneous wounds, linked to its potent anti-inflammatory activity. The electrospinning technique can be used to introduce the desired therapeutic agents to the nanofiber matrix. So here we introduce a new material for wound tissue dressing, in which a polyurethane–dextran composite nanofibrous wound dressing material loaded with β-estradiol was obtained through electrospinning. Dextran can promote neovascularization and skin regeneration in chronic wounds. This study involves the characterization of these nanofibers and analysis of cell growth and proliferation to determine the efficiency of tissue regeneration on these biocomposite polymer nanofibrous scaffolds and to study the possibility of using it as a potential wound dressing material in the in vivo models.

1. Introduction

There has been a rapid growth in the magnitude of elderly population mainly due to the recent advancements in the healthcare management and due to the various epidemiological factors. Along with this scenario, there has an increase in morbidity associated with age-related delayed wound healing. Generally the wounds heal through a multifaceted series of overlapping tightly controlled stages. As we became older, these complex processes were disturbed and repair efficiency was reduced. According to the previous reports, in elderly women the estrogen hormone level in the body quickly reduced after the menopause and this might contributed towards to delay in wound healing. Estrogens possess a considerable role in wound repair that can reverse the reduced wound healing process. Those who were at the post-menopausal condition possess an increased risk of developing an excessive inflammation condition, mainly due to a number of deteriorating pathological properties [1,2]. During the menopause condition, considerable changes to the normal female skin have been occurred. The profound changes were the decrease in dermal collagen and reduced skin thickness and both of which can be overturned by topical estrogen application [3,4]. According to the studies, there exist a strong correlation between the systemic hormone levels and wound healing. It has been reported that the women at the post-menopausal stage taking systemic hormone replacement therapy heal wounds more effectively than the control ones [5].

The cutaneous wound healing is a complicated process linked with an initial inflammatory response, restoration of the epithelial barrier and matrix deposition. The improved deposition of cell matrix, prompt re-epithelialization process, and the potential anti-inflammatory activity can be achieved by the systemic estrogen replacement and which can contribute to accelerate the healing of acute cutaneous wounds [6,7]. In this concern, hormone replacement therapy (HRT) can be a good substitute to prevent the development of chronic wounds in post-menopausal women [8,9]. According to the studies, the exogenous treatment of estrogen can

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reverses the delayed cutaneous wound healing by inhibiting unnecessary neutrophil recruitment, endorsing re-epithelialization and increasing collagen deposition [10,11].

Dextran is a biocompatible, biodegradable, non-immunogenic and non-antigenic biopolymer and hence it has been widely applied in various biomedical applications, such as drug delivery applications [12], as scaffolds for tissue engineering applications [13,14] and as molecular arms [15], etc. Scaffold materials made up of dextran were soft and flexible and hence it improves the handling efficiency for the management of wound treatment [16]. Moreover the dextran-based hydrogel materials have been used as scaffolds for neovascularization and re-epithelialization in wound tissue engineering. In the preparation aspect, dextran is soluble in both water and organic solvents make it as a fine material for bio-applications. So the dextran can be directly blended with suitable polymers such as PU to prepare composite nanofibrous membranes by electrospinning [17]. The physical and biological properties of dextran can be thus manipulated according to the application and hence better biocompatibility can be achieved [18].

The precise adherence to the wound location and good exudate absorbance should be the promising factors of a perfect wound dressing material. They should also retain appropriate moisture along with easy practice and removal [19,20]. Electrospun nanofibers were found to be very effective to be used as the wound dressing material mainly due to its architectural superiority. The electrospun nanofiber can mimic the extra cellular matrix (ECM) environment which will help the host cells to grow and create a new natural cellular matrix [21,22]. Most importantly the preferred therapeutic materials can be introduced to the nanofiber matrix using the electrospinning process [17,23,24], that can seriously affect the wound healing.

We introduce a new PU-dextran based nanofibrous material for potential wound dressing utilizing β-estradiol, the most bioactive endogenous estrogen. In this preliminary work, a composite nanofibrous wound dressing material loaded with β-estradiol was fabricated through electrospinning. The continuous systemic estrogen release from the Estradiol loaded nanofibrous mat at the wound area can accelerate healing of acute cutaneous wounds, linked to its potent anti-inflammatory activity. This preliminary study involves the fabrication, characterization of these composite nanofibers and analysis of cell growth and proliferation to determine the efficiency of tissue regeneration on these biocomposite polymer nanofibrous scaffolds and to study the possibility of using it as an effective wound dressing material in the *in vivo* models.

2. Experimental procedure

2.1. Materials and methods

Polyurethane, 10 wt% (PU, Estane® Skythane® X595A-11, Lubrizol) was prepared by dissolving in solvent mixture of Dimethyl sulfoxide (DMSO, Sigma Aldrich, Korea) and Tetrahydrofuran (THF, Sigma Aldrich, Korea) (50/50, wt:wt%). 5 wt% The Dextran (from Leconostoc mesenteroides, average M_w = 8500–11500, Sigma Aldrich) has been added to those respective solutions along with 2 wt% β-estradiol (Sigma Aldrich, Germany). The obtained solutions were placed in a plastic syringe tube and fed through a metal capillary (nozzle) with a diameter di = 0.21 mm (21 G) attached to a 1-D robot-system that moves laterally and is controlled by the LabVIEW 9.0 software program (National Instrument). The feeding rate was maintained at 0.5 mL/h via a controllable syringe pump. Electrospinning was carried out at a voltage of 18 kV and working distance of 15 cm at room temperature. After electrospinning the mats were carefully removed and kept at overnight vacuum drying to remove the residual solvents.

2.2. Characterizations

The morphology of the electrospun composite mats was observed by using field-emission scanning electron microscopy (FE-SEM, Hitachi S-7400, Hitachi, Japan). The bonding configurations of the samples were characterized by means of Fourier-transform infrared (FT-IR) spectroscopy. Contact angle (wettability) was measured by using the deionized water contact angle measurement system, using contact angle meter (Digidrop, GBX, France). Deionized water was automatically dropped (drop diameter 6 μm) onto the mat.

2.3. Platelet activation study and whole blood clotting assay

The blood clotting studies were done based on reported literature [25]. Blood was mixed with anticoagulant agent acid citrate dextrose at a ratio of 9:1. Later blood was added to each composite nanofiber mats and placed in a 25-mL plastic Petri dish, which was followed by the addition of 10 μL of 0.2 M $CaCl_2$ solutions to initiate blood clotting and PU mat was used as negative control. These mats were then incubated at 37 °C for 15 min. 1 mL of distilled water was then added drop wise without disturbing the clot. Subsequently, 1 mL of solution was taken from the dishes and was centrifuged at 1000 rpm for 1 min. The supernatant was collected for each sample and kept at 37 °C for 1 h. Two hundred microliters (200 μL) of this solution was transferred to a 96-well plate. The optical density was measured at 530 nm using a plate reader (Dynex Technologies, USA).

For platelet activation studies blood mixed with anticoagulant was centrifuged at 2500 rpm for 10 min. The supernatant rich in platelet plasma was collected and added directly on to the nanofibers. Samples were fixed with glutaraldehyde and then washed with PBS. Later these samples were dehydrated using gradient alcohol treatment and then viewed under SEM.

2.4. In vitro drug release study

Estradiol loaded composite nanofibers (2 × 2 cm) were immersed into eppendorf tubes containing 3 mL of phosphate buffer solution (pH 7.4) at 37 °C with continuous shaking at 120 rpm. A fixed volume of the release medium was withdrawn at continuous intervals and replenished with the same volume of the fresh PBS solution. All samples were prepared in triplicate and analyzed using UV–vis.

2.5. Cytocompatibility study

The viability of cultured 3T3-L1 fibroblasts (preadipocytes, Korean Cell Line Bank, Korea) was monitored on the third and sixth day of culture using the colorimetric MTT assay (Sigma, USA). The nanofiber scaffolds were washed twice with PBS and were then treated with approximately 50 μL of the MTT solution (DMEM); the scaffolds, after mixing of the contents by side-tapping, were incubated at 37 °C for 2 h. The nanofiber scaffolds containing MTT-cell mixtures were gently rocked to deposit the cells. The supernatant of the MTT solution was pipette out and then acid–isopropanol (95 mL isopropanol with 5 mL 3 N HCl) was added to the colored cell deposit. After gently mixing the acid–alcohol-treated scaffolds, it was then allowed to react for 5 min. 100 μL of the purple–blue colored supernatant that contained the solubilized formazan in each sample was added to a well in a 96-well plate for analysis at 580 nm in an ELISA reader. The cell viability was obtained by comparing the absorbance of cells cultured on the nanofibers scaffold to that of control well containing cells. The results were expressed as the mean ± standard error of the mean. The data were analyzed via the Student's *t* test and repeated measures of analyses of variance