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A bioengineered drug-Eluting scaffold accelerated cutaneous wound healing In diabetic mice

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ABSTRACT

Hyperglycemia in diabetic patients can greatly hinder the wound healing process. In this study we investigated if the engagement of F4/80$^+$ murine macrophages could accelerate the cutaneous wound healing in streptozotocin induced diabetic mice. To facilitate the engagement of macrophages, we engineered a drug-eluting electrospun scaffold with a payload of monocyte chemoattractant protein-1 (MCP-1). MCP-1 could be readily released from the scaffold within 3 days. The electrospun scaffold showed no cytotoxic effects on human keratinocytes *in vitro*. Full-thickness excisional cutaneous wound was created in diabetic mice. The wound fully recovered within 10 days in mice treated with the drug-eluting scaffold. In contrast, the wound took 14 days to fully recover in control groups. The use of drug-eluting scaffold also improved the re-epithelialization. Furthermore, we observed a larger population of F4/80$^+$ macrophages in the wound bed of mice treated with drug-eluting scaffolds on day 3. This marked increase of macrophages in the wound bed could have contributed to the accelerated wound healing. Our study shed new light on an immuno-engineering solution for wound healing management in diabetic patients.

1. Introduction

Approximately 15% of diabetic patients suffer impaired wound healing, especially foot ulcers [1]. It was reported that foot ulceration was a preceding event in 85% of diabetic patients who had lower limb amputations [2,3]. The cutaneous wound healing is a highly complex process, involving a number of growth factors and cellular events [4]. The hyperglycemia in diabetic patients can lead to neuropathy, vascular dysfunction and other complications that can significantly impair the wound healing process [5].

In recent years the role of immunological cells in the wound healing process has garnered an increasing attention. The wound healing process can be broadly divided into four stages, that is, the hemostasis stage, the inflammatory stage, the proliferative stage and the remodeling stage [6]. Following the cutaneous injury, monocytes will populate the wound bed *via* blood circulation and differentiate into macrophages, which is followed by phagocytosis, angiogenesis and re-epithelialization, *etc.* [7,8]. Consequently, a boosted recruitment of monocytes into the wound bed is supposed to lead to an accelerated wound healing.

Recently tissue-engineering scientists have rigorously investigated how to manipulate the immune system for therapeutic purposes. Previous research showed that nanomaterials could be programmed to solicit specific immune responses [9,10]. In addition, nanomaterials with a pharmaceutical payload could perturb the immune system, for example, the polarization of macrophages [11].

Previous research demonstrated that wound dressing made of tissue-engineered scaffolds could promote wound healing [12,13]. For example, diabetic wounds treated with electrospun scaffolds with plasmid human epidermal growth factor showed a marked improvement of wound healing [14]. Also, an increased accumulation of neo-collagen and cytokeratin was observed. These previous studies established that an electrospun scaffold could be used as an effective delivery vehicle for therapeutic purposes in diabetic mice. Consequently, in our study, we explored how to use an electrospun scaffold with a payload of monocyte chemoattractant protein-1 (MCP-1) to accelerate the cutaneous wound healing process in diabetic mice.

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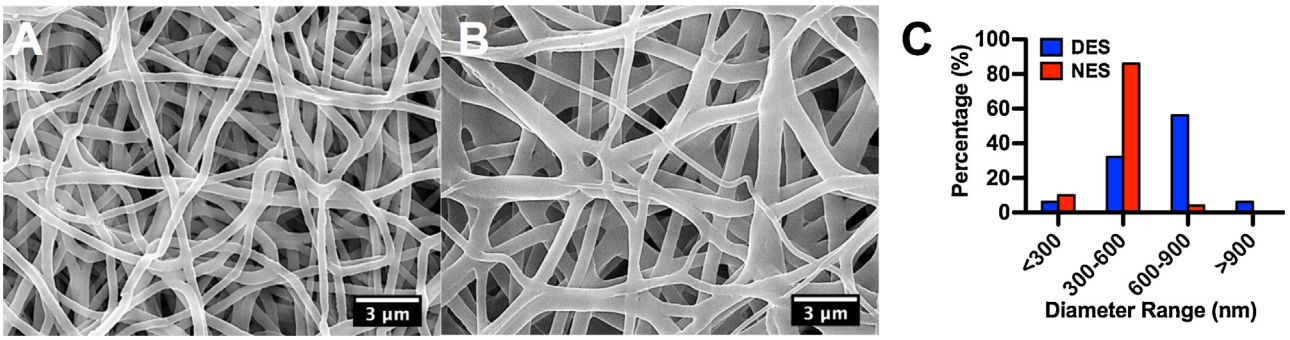

Fig. 1. SEM images of scaffolds. (A) NES; (B) DES; (C) fiber diameter distribution both scaffolds were composed of randomly distributed non-woven fibers. NES: non-eluting scaffold. DES: drug-eluting scaffold.

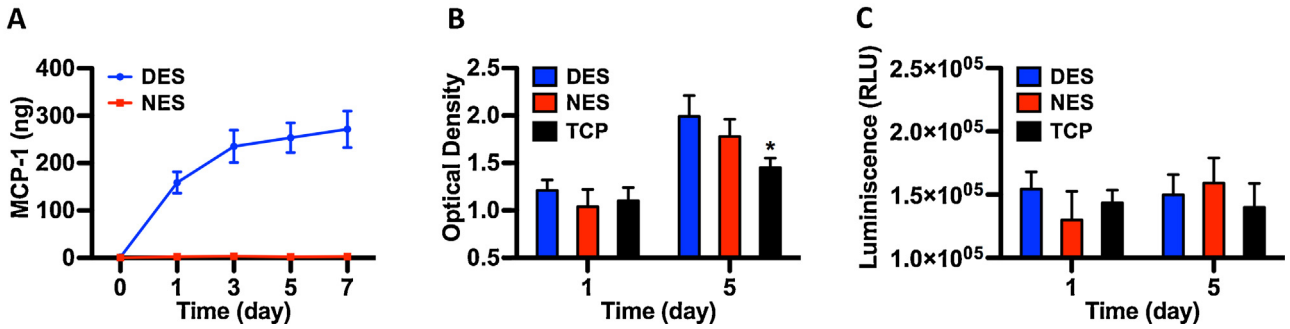

Fig. 2. Biophysical characterizations of scaffolds. (A) pharmacokinetics of MCP-1 release; (B) the viability of keratinocytes; (C) the apoptosis of keratinocytes. The majority of MCP-1 was released within 3 days. Keratinocytes readily proliferated on electrospun scaffolds, outgrowing their peers on TCP, and suffered no apoptosis. These results suggested that the electrospun scaffold provided a favorable substrate for the proliferation of keratinocytes. Also, the scaffolding materials showed no cytotoxicity. NES: non-eluting scaffold; DES: drug-eluting scaffold; TCP: tissue culture plate. n = 3 in all experiments. ANOVA with Tukey test was performed. The error bar denotes standard deviation.

2. Materials and methods

2.1. The construction of electrospun scaffolds

Bovine gelatin (Sigma Aldrich, St. Louis, MO) and polyglycolic acid (PGA) (Sigma Aldrich, St. Louis, MO) were purchased and dissolved in 1,1,1,3,3,3-Hexafluoro-2-propanol (HFP) (Sigma Aldrich, St. Louis, MO) (weight ratio = 5:1) at room temperature for 24hr with continuous stirring to obtain a homogeneous solution. The solution was loaded into a syringe with a 27-gauge needle distanced at 25 cm from a collector. 2 ml of the solution was electrospun onto the collector at a voltage of 30 kV. The electrospun scaffold was retrieved from the collector and desiccated in vacuum for at least 24 h. For drug-eluting scaffold, MCP-1 (Peprotech, Rocky Hill, NJ, USA) was completely dissolved in the gelatin/PGA solution (6 μg/ml) and then electrospun under the same set of conditions. Prior to subsequent studies, all scaffolds were sterilized in 70% ethanol followed by extensive rinse in sterile PBS buffer.

2.2. The in vitro characterizations of the scaffolds

To characterize the morphology of electrospun fibers in the scaffold, square samples (1 cm × 1 cm) of both non-eluting and drug-eluting scaffolds were sputter-coated with gold and then studied using scanning electron microscopy (SEM). The diameter of fibers in respective scaffolds was measured using ImageJ. The porosity of respective scaffolds was calculated as previously reported [15].

To study the pharmacokinetics of MCP-1 release, the drug-eluting scaffolds (D = 5 mm) were incubated in keratinocytes culture media for up to 7 days. The theoretical dose loading of each scaffold sample was 382 ng. The supernatant was collected on day 1, 3, 5 and 7 for enzyme-linked immunosorbant assay (ELISA) (R&D Systems, Minneapolis, MN) per manufacturer's protocols. The scaffolds were incubated in keratinocytes culture media because keratinocytes were used in subsequent viability and apoptosis analyses. Non-eluting scaffolds were used as control.

Primary adult human keratinocytes (Life Technologies, Grand Island, NY) were used to evaluate the cytotoxicity of scaffolding materials. Keratinocytes were seeded on top of non-eluting scaffolds, drug-eluting scaffolds (177 cells/mm^2) and each well in a 96-well tissue culture plate (TCP) (control). Keratinocytes were cultured for 5 days at 37 °C and 5% CO_2. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) kit and Caspase-Glo® 3/7 kit were used per manufacturers' protocols to measure the viability and apoptosis in keratinocyte populations, respectively, on day 1 and 5.

2.3. The creation of cutaneous wound in diabetic mice

A single intraperitoneal injection of streptozotocin (STZ) (100 mg/kg) (Sigma Aldrich, St. Louis, MO) was used to induce diabetes in female C57BL/6 mice (8–10 weeks old). Non-fasted mice whose blood glucose level exceeded 250 mg/dl for two consecutive days were considered diabetic and used for *in vivo* studies. According to previous research, STZ-induced diabetic mice suffer from a range of side effects, which would lead to a limited lifespan after the induction [16,17]. To ensure a minimum of two-week lifespan without significant side effects after the induction, we chose 250 mg/dl as the threshold for being considered diabetic based on previous studies. Anesthesia was administered in diabetic mice using vaporized isofluorane. Hairs on the dorsal area were completely clipped and the skin thoroughly sanitized. A full thickness excisional cutaneous wound (D = 5 mm) was created on the dorsal area of each