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Biological function evaluation and effects of laser micro-pore burn-denatured acellular dermal matrix

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ABSTRACT

Objective: In the field of burns repairs, many problems exist in the shortage of donor skin, the expense of allograft or xenograft skin, temporary substitution and unsatisfactory extremity function after wound healing. Previous studies showed that burn-denatured skin could return to normal dermis formation and function. This study investigates the application of laser micro-pore burn-denatured acellular dermis matrix (DADM) from an escharotomy in the repair of burn wounds and evaluates the biological properties and wound repair effects of DADM in implantation experiments in Kunming mice.

Methods: Specific-pathogen-free (SPF) Kunming mice were used in this study. A deep II° burn wound was created on the dorsum of the mice by an electric heated water bath. The full-thickness wound tissue was harvested. The necrotic tissue and subcutaneous tissue were removed. The denatured dermis was preserved and treated with 0.25% trypsin, 0.5% Triton X-100. The DADM was drilled by laser micro-pore. The biological properties and grafting effects of laser micro-pore burn-DADM were evaluated by morphology, cytokine expression levels and subcutaneous implantation experiments in Kunming mice.

Results: We found statistical significance ($P<0.05$) of the elastic modulus (MPa), maximum load force (N) and contraction measurement (CM) of the laser micro-pore burn-DADM (experimental group) compared to the control group (no laser micro-pore burn-DADM). Cytokine expression level was different in the dermal matrixes harvested at various time points after burn (24h, 48h, 72h and infected wound group). Comparing the dermal matrix from 24h burn tissue to infected wound tissue, the expression level of IL-6, MMP-24, VE-cadherin and VEGF were decreased. We found no inflammatory cells infiltration in the dermal matrix were observed in both experimental and control groups (24h burn group), while the obviously vascular infiltration and fiber fusion were observed in the experimental group after subcutaneous implantation experiments.

Abbreviations: DADM, denatured acellular dermal matrix; SPF, specific-pathogen-free; VEGF, vascular endothelial growth factor; TCK-1, thymus chemokine-1; MT5-of MMP, membrane-type matrix metalloproteinase-5; MMP-24, matrix metalloproteinase-24; DDM, denatured dermal matrix.

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Conclusion: There was better bio-performance, low immunogenicity and better dermal incorporation after treated by laser micro-pore drilling and decellularized deep II° burn-DADM, which may be considered as a better substitute for dermal matrix. Furthermore, the earlier harvested DADM after burn (24h) shows the better transplantation effect.

1. Introduction

There are some problems in the field of burn repairs which includes shortage of donor skin, the expense of allograft or xenograft skin, temporary substitutions and unsatisfactory extremity function after wound healing [1]. In our previous studies, we found there is a special layer of dermal tissue which was not burned to necrosis in the deep II° and mixed degree burned skin, in which the cells are disorders and dysfunction. The morphology of this special layer of dermal tissue is abnormal, but with improvement of local environment, the special layer of dermal tissue could recover to normal morphology and function [2]. Research showed systemic application of P188 in the deep II° and the burn-denatured skin could return to normal dermis formation and function in the early burn stage [3]. Our previous study showed that the deep II° burn-denatured dermal matrix treated with 0.25% trypsin and 0.5% Triton X-100 has lower immune rejection and better tissue compatibility after implantation in animal [2,4]. However, after the thermal injury, the burn toxins are released and can induce adverse effects in patients [2]. Furthermore, denatured dermal matrix has the following issues that need to be resolved, including tissue fragility and "toxin" residue. Vascular infiltration and cells growth slowly were also observed in implantation studies [4]. Study shows that laser micro-pore PADM grafting in combination with split-thickness autografting can improve wound healing [5]. We applied a laser micro-pore technique on deep II° burn-denatured dermis, after that the laser micro-pore burn-DADM was treated with trypsin and Triton-100. The biological properties and grafting effects of laser micro-pore burn-DADM were evaluated by examining the properties and cytokine level and performing an implantation experiment in Kunmimg mice.

2. Materials and methods

2.1. Laboratory animal selection and preparation of DADM

A total of 60 health specific-pathogen-free (SPF) Kunming mice, aged 12–14 weeks and weighing 40±3g, were housed in the standard animal environment. The mice were allowed to adapt to the environment of the lab for 5–7days and were randomly selected to receive a deep II° burn wound. All animals were purchased from Experimental Animal Center of Shandong University. The certificate number was SCXK (Lu) 20150002. These mice were raised in accordance with the Guidelines for the Care and Use of Laboratory Animals published by National Institutes of Health of the USA (1996 Revision).

2.2. Establishment of the animal model

First, 10% chloral hydrate (30mg/kg) was used to anesthetize the animals. After successful anesthesia, the experimental animal was secured in a stationary position. Freshly prepared depilatory were evenly applied on the dorsum to completely wet the hair. After 3s, the hairs were removed, sterile saline was used to wipe off the depilatory, and this skin area was dried.

An electric heated water bath was used to control the water temperature. The temperature to make a burn injury was set at 90°C. The surgeon wore thick waterproof gloves. The animal was secured in a stationary position, and its dorsum was immersed in the hot bath for 12s [2]. The animal was removed from the hot bath immediately after the timer went off. A dry towel was used to dry the dorsum, and then iodophor was applied to the wound site. The injured animal received Ringer's solution (40ml/kg) via intraperitoneal injection for fluid resuscitation. The animal was housed alone after it awoke from the anesthesia. Povidone-iodine was applied to the burn wound twice daily. Adequate care was taken to prevent the animals from biting each other.

2.3. Preparation and grouping of DADM

The animals were sacrificed by cervical dislocation at 24, 48h, 72h after the burn. After povidone-iodine disinfection, the skin at the burn wound was harvested and repeatedly washed in sterile saline. Using aseptic technique, the subcutaneous fascia, fat and muscles were trimmed and removed. The deep II° burn skin tissues were selected for this study by histological examination (H&E) for a small piece of skin, and the remaining skin tissues were stored at 4°C for future use.

The deep II° burn skin tissues were soaked in a 0.1% benzalkonium bromide solution for 30min and then cut into 3×2-cm^2 DADM sheets, which were placed in a 250ml jar with 0.25% trypsin and 0.5% Triton X-100 for 15min. The solution was titrated to 100ml with PBS. The jar was put on a 37°C thermostat shaker at 100rpm vortex for 2h. After vortex, the DADM sheets were washed in PBS for 12h. The PBS was replaced with fresh solution every 6h until the PBS in the jar was completely clear. The DADM sheets were randomly divided into two groups: the experimental group and the control group. The specific laser template drilling technique was then used to produce a total of 300 micro-holes in a 3×2-cm^2 portion of the dermal matrix sheet under laser template guidance. The diameter of the micro-holes is 135μm, and the distance between the micro-holes is 1mm [6].

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