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Sustainable antimicrobial effect of silver sulfadiazine-loaded nanosheets on infection in a mouse model of partial-thickness burn injury

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ABSTRACT

Partial-thickness burn injury has the potential for reepithelialization and heals within 3 weeks. If the wound is infected by bacteria before reepithelization, however, the depth of disruption increases and the lesion easily progresses to the full-thickness dermal layers. In the treatment of partial-thickness burn injury, it is important to prevent the wound area from bacterial infection with an antimicrobial dressing. Here, we have tested the antimicrobial properties of polymeric ultra-thin films composed of poly(lactic acid) (termed "PLA nanosheets"), which have high flexibility, adhesive strength and transparency, and silver sulfadiazine (AgSD), which exhibits antimicrobial efficacy. The AgSD-loaded nanosheet released Ag^+ for more than 3 days, and exerted antimicrobial efficacy against methicillin-resistant *Staphylococcus aureus* (MRSA) in an *in vitro* Kirby–Bauer test. By contrast, a cell viability assay indicated that the dose of AgSD used in the PLA nanosheets did not show significant cytotoxicity toward fibroblasts. *In vivo* evaluation using a mouse model of infection in a partial-thickness burn wound demonstrated that the nanosheet significantly reduced the number of MRSA bacteria on the lesion (more than 10^5-fold) and suppressed the inflammatory reaction, thereby preventing a protracted wound healing process.

1. Introduction

Bacterial infection is one of the most critical issues in burn injuries. Burn injury leads to a skin defect—that is, a loss of the physical barrier against exogenous microbial invasion—rendering the host susceptible to bacterial infection. In the case of partial-thickness burn injury, the epidermis and part of the dermis are disrupted; therefore, wound lesions reepithelize in several days and completely heal within 3 weeks [1]. If the wound is infected by bacteria during reepithelization, however, the disruption depth increases and the injury easily progresses to the full-thickness dermal layers, which then require skin grafting in order to heal without scarring [2]. Therefore, antimicrobial wound dressings are required to control infection in a partial-thickness burn wound in order to inhibit microbial growth in the lesion during the early stage of reepithelization and to prevent the disruption depth increasing.

In the treatment of a full-thickness burn wound, the wound should be protected until skin grafting will be performed because natural cure is not expected. However, a partial-thickness burn wound can be cured naturally by protecting and inhibiting the progression for several days. Therefore the strategy of protection is different between a full- and a partial-thickness burn. In the treatment of a partial-thickness burn wound, especially a bacterially infected wound, antimicrobial potency is appropriately achieved by using wound dressings containing antimicrobial agents such as silver within a hydrocolloid film (Urgotul SSD, Urgo) [3], a hydrofiber (Aquacel Ag, Conva Tec) [4], or a polyurethane film (PolyMem Silver, Ferris) [5]. Silver compounds are widely commercialized as broad-spectrum antimicrobial agents [6].

In recent years, multi-drug-resistant bacteria, such as methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus* (VRE), have evolved with resistance to many antibiotics and have become a major cause of bacterial infection [7]. In this regard, wound dressings containing silver compounds are used clinically in many countries due to their effectiveness against multi-drug-resistant bacteria. However, such wound dressings are unstable on the wound lesion owing to their

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weak adhesive properties, and follow-up observation after treatment is difficult because none of the current dressings is transparent. Antimicrobial creams (e.g. Geben cream, Tanabe Mitsubishi Pharma Co.) are also commonly used for infected partial- or full-thickness burn wounds [8]; however, their stickiness to cloth and the daily care needed such as washing and application are drawbacks. Therefore, improved adhesiveness for longer period to lesions rather than to cloth and transparency would simplify the treatment and reduce the burden for patients.

To this end, we are interested in polymeric ultra-thin films (termed "nanosheets") as a new candidate for wound dressings. With a thickness of tens of nanometers, nanosheets have unique physical characteristics such as high flexibility, strong adhesiveness, and high transparency due to their ultra-thin structure. A nanosheet was adhered directly over the wound area and then covered with a mesh film and an occlusive dressing for protection. The transparent nanosheet covering on the wound area allowed us to observe wound recovery by simply removing and replacing the protecting dressings. We have previously reported their potential utility in biomedical applications [9–16]. Moreover, nanosheets are easily adhered to biological surfaces such as the skin [9], lung [10], stomach [11], and cecum [12] without chemical glue. Therefore, nanosheets composed of biocompatible and biodegradable polymers (e.g., poly(lactic acid); PLA) are expected to be highly applicable as wound dressing materials. In fact, we previously demonstrated that PLA nanosheets can be utilized as an alternative to suturing, resulting in little scar formation after gastric incision [11]. Furthermore, PLA nanosheets protected wound areas and prevented bacterial infection in a partial-thickness burn wound [13]. During the course of burn-wound treatment with PLA nanosheets, cumbersome daily care such as renewing the dressing coupled with careful washing can be reduced because the PLA nanosheet is adhered directly to the burn wound, where it maintains its antimicrobial effect for more than three days and degrades within a few weeks. More recently, we introduced drug-loaded nanosheets, and demonstrated that an antibiotic tetracycline-loaded nanosheet showed clinical utility for perforative peritonitis and full-thickness burn wounds [14,15].

We previously reported that PLA nanosheets have a unique membrane permeability [16], relating to the film thickness and crystallinity. In this study, we report the development of drug-loaded PLA nanosheets with a sustainable release property lasting more than 3 days based on the molecular permeability of the nanosheet. For the silver agent, we selected silver sulfadiazine (AgSD), one of the topical antimicrobial silver agents clinically used for burn wound [17,18] that has antimicrobial efficacy against multi-drug resistant bacteria such as MRSA [19]. Although researchers have described antimicrobial materials based on AgSD for burn wound treatment [20–23], silver agents are cytotoxic and AgSD is no exception [24]. Therefore, it was important to reduce the risk of cytotoxicity by decreasing the content of AgSD. Specifically, we sandwiched a small amount of AgSD particles between two layers of PLA nanosheets. This structure has two functions: first, physical fixation of AgSD particles on the wound site; and second, controlled release of Ag^+. We evaluated the Ag^+-release properties and the sustainability of antimicrobial efficacy, cell viability, and also evaluated the utility of the AgSD-loaded nanosheet as a wound dressing material for a mouse model of infection in a partial-thickness burn wound.

2. Materials and methods

2.1. Materials and animals

Biocompatible poly-(DL-lactic acid) (PLA; Mw, 300,000–600,000) was purchased from Polyscience Inc. (Warrington, PA). Poly-(vinyl alcohol) (PVA, Mw; 22,000) was purchased from Kanto Chemical Co., Inc. (Tokyo, Japan). Silver sulfadiazine (AgSD) was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Silicon wafers purchased from KST World Co. (Fukui, Japan) were cut to an appropriate size, immersed in a mixture of sulfuric acid and hydrogen peroxide (3/1) for 10 min, and then thoroughly rinsed with D.I. water (resistivity 18 MΩ cm). Male C57BL/6 mice (8 weeks old, weighing 22–25 g; Japan SLC, Hamamatsu, Japan) were used in experiments in accordance with the guidelines of the Institutional Review Board for the Care of Animal Subjects at the National Defense Medical College, Japan.

2.2. Preparation of AgSD-loaded nanosheets

The AgSD-loaded nanosheets were fabricated by the following method (Fig. 1) in a clean room (class 10,000 conditions) to avoid

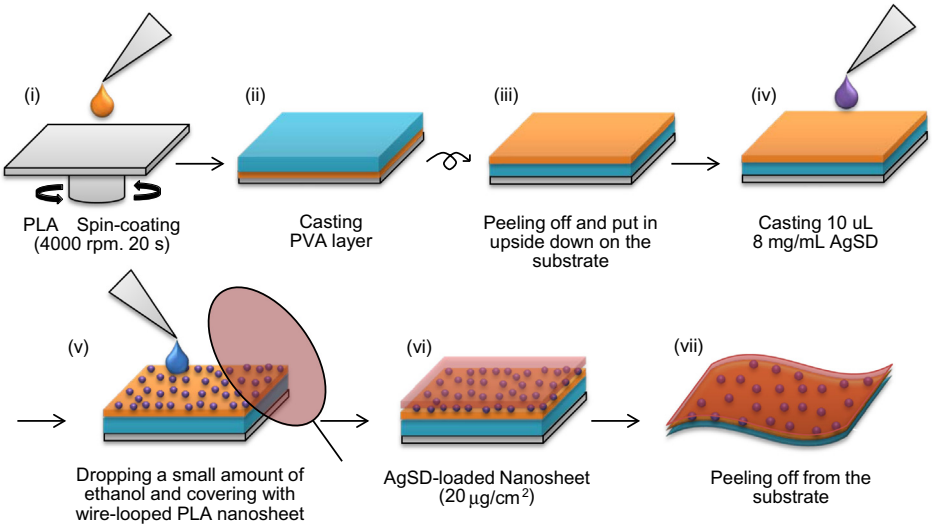

Fig. 1. Scheme for the preparation of AgSD-loaded nanosheets. (i) PLA was spin-coated on the SiO_2 substrate. (ii) PVA was cast on the protection layer. (iii) After the PVA supporting film was dried, the protection layer was peeled off with the PVA film and reversed onto the substrate. (iv) AgSD solution was deposited onto this layer and particles were crystallized by evaporating the solvent. (v) A small amount of ethanol was dropped on the layer, and the AgSD particles were covered with the release layer by means of a wire loop. (vi) The AgSD-loaded nanosheet was prepared on the substrate. (vii) The AgSD-loaded nanosheet was easily peeled off from the substrate via the PVA supporting film.

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