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Wound healing potential of antibacterial microneedles loaded with green tea extracts

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ABSTRACT

This study evaluates the utility of an antibacterial microneedle composed of green tea (GT) extract and hyaluronic acid (HA), for the efficient delivery of GT. These microneedles have the potential to be a patient-friendly method for the conventional sustained release of drugs. In this study, a fabrication method using a mold-based technique to produce GT/HA microneedles with a maximum area of ~50 mm^2 with antibacterial properties was used to manufacture transdermal drug delivery systems. Fourier transform infrared (FTIR) spectrometry was carried out to observe the potential modifications in the microneedles, when incorporated with GT. The degradation rate of GT in GT/HA microneedles was controlled simply by adjusting the HA composition. The effects of different ratios of GT in the HA microneedles were determined by measuring the release properties. In HA microneedles loaded with 70% GT (GT70), a continuous higher release rate was sustained for 72 h. The in vitro cytotoxicity assays demonstrated that GT/HA microneedles were not generally cytotoxic to Chinese hamster ovary cells (CHO-K1), human embryonic kidney cells (293T), and mouse muscle cells (C2C12), which were treated for 12 and 24 h. Antimicrobial activity of the GT/HA microneedles was demonstrated by ~95% growth reduction of gram negative [*Escherichia coli* (*E. coli*), *Pseudomonas putida* (*P. putida*), and *Salmonella typhimurium* (*S. typhimurium*)] and gram positive bacteria [*Staphylococcus aureus* (*S. Aureus*) and *Bacillus subtilis* (*B. subtilis*)], with GT70. Furthermore, GT/HA microneedles reduced bacterial growth of infected wound sites in the skin and improved wound healing process of skin in rat model.

1. Introduction

Transdermal delivery systems using microneedles with antibacterial properties are used for controlled, consistent, and pain-free administration of therapeutic drugs to a patient's skin for wound healing [1–3]. Microneedle arrays are needle-like structures with diameters in the order of microns, which are used to disrupt the outer layers of the skin to enable (trans)dermal drug delivery [4]. Microneedle arrays have been developed using various materials, including silicon [5], glass [6], metal [7], and polymers [8], and with a variety of shapes and sizes, as needed for different applications. However, silicon, glass and metal, microneedles can result in sharp bio-hazardous wastes with immunogenic consequences. Among these materials, biodegradable polymer microneedles are also of interest for (trans)dermal drug delivery due to their enhanced biocompatibility and capability to encapsulate the drug within the microneedle matrix [9,10]. Various materials such as sugars [11] and water-soluble polymers [10] have been used to fabricate dissolvable microneedles. In these cases, the microneedles gradually dissolve or degrade in the skin, releasing the loaded drugs, and the dressing materials can be easily removed after the healing process [12,13]. Compared to coated microneedle systems, dissolvable microneedles can incorporate large amounts of drugs and prevent potential immunogenic reactions to the microneedles during drug delivery [14–16].

One of the approaches to treat various types of wound infections is to reduce the bacterial load in the wound [17,18]. An infection can seriously delay wound healing. As an antibacterial agent, green tea (GT)

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extract has drawn considerable attention owing to its ability to prevent infections. The major compounds present in GT include polyphenols, which are potent antibacterial, anti-carcinogenic, anti-viral, anti-allergic, and anti-inflammatory agents [19,20]. Catechins present in GT have shown inhibitory effects on gram-positive and gram-negative bacteria by disrupting cell membranes and decomposing essential metabolites [19–22].

Here, we report the fabrication of bio-polymer (hyaluronic acid; HA)-based large-area microneedles in different shapes/formats, which facilitate the loading and release of GT for controlled antibacterial molecule delivery. Our dissolvable microneedle arrays are composed of HA as the base material. As HA is a polysaccharide, which is commonly present in most species and localized in multiple sites of the human body, including the skin and soft tissue, it is a safe exogenous material for insertion. In addition, its low cost, biodegradability, and non-polluting nature make it a suitable candidate for wound dressing [23,24]. These HA microneedles loaded with green tea extract (GT/HA) can be an attractive approach for topical treatment of skin infections by the minimally invasive delivery of antimicrobial molecules. GT has a strong capability to kill bacteria, and its activity persists even when it is incorporated into a polymer matrix. The major functional advantage of GT/HA microneedles is the reduction of bacterial colonization. They provide a safe barrier to protect the wound from further physical damages and any contaminations from exogenous organisms. In addition, the molding processes make it easy to impregnate the various structures of microneedles with antibacterial or therapeutic agents. These systems are superior to conventional microneedle systems with controllable drug delivery and have significant potential for novel clinical applications.

2. Experimental section

2.1. Preparation of GT

Dried GT leaves before chemical treatment were purchased from Boseong Co. Ltd., Korea. The GT leaves were steamed and parched after picking to prevent oxidation of the catechins as the main compound present in the leaf. The dried leaves (100 g) were mixed with distilled water (DI water; 1 L) and heated at 80 °C for 30 min with continuous stirring, followed by the addition of absolute ethanol (300 mL) at room temperature to give 24 h extraction. The decanted supernatant was filtered twice through Whatman® No. 4 filters. The GT solution was placed in a freezer at −80 °C for one day and then dried in a freeze dryer (−42 °C, below 133×10^{-3} mbar) for 48 h. After freeze-drying, the samples were pulverized to obtain a powdered form.

2.2. Fabrication of GT/HA microneedles

Fig. 1 illustrates the process employed to fabricate GT/HA microneedles for wound healing. HA and green tea extract (GT) were mixed in DI water for more than 0.5 h at room temperature to obtain mixtures at different ratios of GT [0% (HA), 10% (GT10), 30% (GT30), 50% (GT50) and 70% (GT70)]. The mixed solution was cast on the master at high pressure to remove bubbles. Then, the solution was loaded into the press mold by drop casting. Dissolvable GT/HA mixture microneedles were created via in situ polymerization of liquid monomer within a press mold. The consistent pressing force is 2 kgf/cm^2 at a temperature of 50 °C for 5 min. During the casting process, the mixture was continuously compressed to minimize void formation. The microneedles were then heated for crosslinking (press) and hardened in a dry oven at 60 °C for 2 h. The cured GT/HA microneedles were subsequently peeled from the master structures. Finally, the GT/HA microneedle array was gently peeled out of the mold.

2.3. Characterization of GT/HA microneedles

Arrays of 50 × 50 mm^2 wedge type microneedles were made with 250 μm radius tips, and 500 μm height (tip-to-tip distance of all samples was 1 mm). The resulting GT/HA microneedles were examined using a scanning electron microscope (SEM, Hitachi S-4000, Tokyo, Japan). Fourier transform infrared (FTIR) spectrometry was carried out to observe the structural interactions of microneedles incorporated with GT. The FTIR spectra of GT/HA microneedle were recorded from 400 to 4000 cm^{-1} at a resolution of 1 cm^{-1} using a Nicolet Magna IR 550 FTIR spectrometer (Nicolet Instrument Corporation, Madison, WI, USA).

2.4. Antibacterial biomolecules release test of GT/HA microneedle into the liquid culture medium

To quantify the release kinetics of GT/HA microneedles [25], samples were prepared by the immersion of microneedles of different GT

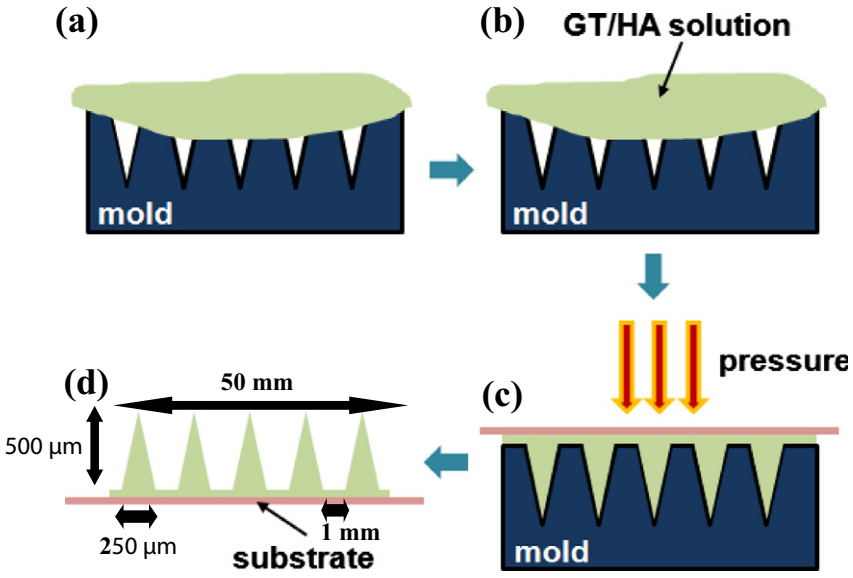

Fig. 1. Illustration of the fabrication process for biodegradable microneedles. (a) preparation of press mold, (b) cover of green tea extract and hyaluronic acid (GT/HA) solution, (c) filling of GT/HA solution in the cavities of press mold using master structure, and (d) GT/HA microneedles peeled from the master structures.