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Research Paper

Physicochemical properties of pH-sensitive hydrogels based on hydroxyethyl cellulose–hyaluronic acid and for applications as transdermal delivery systems for skin lesions

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ABSTRACT

We investigated the physicochemical properties of pH-sensitive hydroxyethyl cellulose (HEC)/hyaluronic acid (HA) complex hydrogels containing isoliquiritigenin (ILTG), and discussed potential applications as transdermal delivery systems for the treatment of skin lesions caused by pH imbalance. HA has skin compatibility and pH functional groups and HEC serves as scaffold to build hydrogels with varied HCE:HA mass ratio. Hydrogels were synthesized via chemical cross-linking, and three-dimensional network structures were characterized via scanning electron microscopy (SEM). The swelling properties and polymer ratios of the hydrogels were investigated at pH values in the range 1–13. HECHA13 (i.e., an HEC:HA mass ratio of 1:3) was found to have optimal rheological and adhesive properties, and was used to investigate the drug release efficiency as a function of pH; the efficiency was greater than 70% at pH 7. Antimicrobial activity assays against *Propionibacterium acnes* were conducted to take advantage of the pH-sensitive properties of HECHA13. At pH 7, we found that HECHA13, which contained ILTG, inhibited the growth of *P. acnes*. Furthermore, HECHA13 was found to exhibit excellent permeability into the skin, which penetrated mostly via the hair follicle. These results indicate that this pH-sensitive hydrogel is effective as a transdermal delivery system for antimicrobial therapeutics, with potential applications in the treatment of acne.

1. Introduction

Sensitivity to pH is an important property for transdermal delivery systems. The pH of the skin surface, or stratum corneum (SC), is typically in the range 5.0–6.0. This is termed the 'acid mantle', and the pH is influenced by number of internal and external factors, including epidermal cells, glands (sebaceous, apocrine, and eccrine), age, and gender. Maintaining the pH of the SC in the range 5.0–6.0 is important in providing an effective barrier, as well as maintaining physiological processes, due to the optimal structure of the intercellular lipid and SC homeostasis [1,2]. SC intercellular lipids form a stabilized double lamellar structure in these mildly acidic conditions, whereas micellization occurs for pH >6.0 and a disordered structure occurs for pH <4.5 [3]. This unbalanced pH (break down the acid mantle) decreased the SC cohesion and permeability barrier, and also skin illness occurs such as inflammation, acne and irritant contact dermatitis [1]. Acne is the common form of skin lesion, and is particularly common among teenagers, although it may present at any age [4].

Hydrogels are typically composed of a natural polymer, such as hyaluronic acid (HA), collagen, gelatin, or cellulose, and are three-dimensional networks of hydrophilic polymer chains. Swollen hydrogels absorb large quantities of water without dissolution. Furthermore, hydrogels feature unique mechanical and structural properties, which are similar to those of tissues and to the extracellular matrix (ECM) of the skin. The unique properties of hydrogels, make them suitable for applications as biomaterials for scaffolds in the tissue engineering, drug delivery systems, imaging, therapeutics, and medical devices [5–7]. To exploit the potential of these materials, novel smart hydrogels have been developed by using environmental stimuli-responsive polymers that are designed to respond to environmental factors such as pH, temperature, enzymes, and electric or magnetic fields [8–10]. Smart hydrogels are composed of functional groups on the polymer backbone of the structure, which result from noncovalent bonding, such as hydrogen bonding, hydrophobic interactions, π–π stacking,

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or electrostatic interactions. When smart hydrogels are exposed to environmental factors, the porosity and hydrophilicity of the hydrogel can regulate the loading and release of drug in a controlled manner. Because of this self-regulating behavior, smart hydrogels are promising candidate materials for drug delivery systems [11–13].

HA is a linear polysaccharide composed of repeating units of β-1,4-linked N-acetyl-D-glucosamine and glucuronic acid. The pK_a of the carboxyl groups of the HA is in the range 3–4, and these groups are ionized at pH 7. HA is important in skin, and is involved in regulating cell proliferation, migration, and differentiation in the epidermis. It is a primary constituent of the ECM and controls tissue physiological function in the dermis. Furthermore, HA is capable of binding to peptides, matrix proteins, and growth factors [14–17]. HA is a hydrophilic polymer that can absorb large quantities of water and can contain up to 1000 fold more water than its solid volume, due to formation of the hydrogen bonding between carboxyl, and N-acetyl groups of HA with water. The favorable biocompatibility, lack of toxicity, and biodegradation properties of HA make it suitable for a wide range of medical applications, including ocular medicine, plastic surgery, tissue engineering, and drug delivery. HA is commonly used as an ingredient in cosmetics, provides anti-aging and moisture supplying effects to the skin, and is often used as fillers during cosmetic surgery to fill wrinkles and grooves caused by facial aging [18–21]. Cellulose is a common, naturally occurring polymer of glucose; however, it is insoluble in water, as well as many other organic solvents. Cellulose-based derivatives have been developed to improve the solubility of cellulose, including methylcellulose (MC), hydroxypropyl methylcellulose (HPMC), ethyl cellulose (EC), hydroxyethyl cellulose (HEC), and sodium carboxymethylcellulose (NaCMC) [22].

Isoliquiritigenin (ILTG) is a component of *Glycyrrhiza uralensis*, which is distributed in China and other Southeast Asian countries as a traditional medicine. There have been many reports of beneficial effects of ILTG, including inhibition of the growth of prostate cancer, reduction of prostaglandin E_2 and nitric oxide, and inhibition of tyrosinase [23–26]. ILTG also exhibits antimicrobial activities, which are superior to those of other licorice ingredients (glycyrrhizin, liquiritin, liquiritigenin), against *Bacillus subtilis*, *Propionibacterium acnes*, and *Pseudomonas aeruginosa*. Furthermore, the ethyl acetate fraction of Korean licorice contains more ILTG compared with licorice from other countries, including China and Uzbekistan [27].

In this study, we assessed the physicochemical properties of hydroxyethyl cellulose–hyaluronic acid (HECHA) complex hydrogels containing ILTG, and investigated their suitability for applications in transdermal delivery. Hydrogels were prepared with various HEC:HA mass ratios, and the physicochemical properties were investigated using Fourier transform infrared (FT-IR) spectroscopy, scanning electron microscopy (SEM), thermogravimetric analysis (TGA), swelling ratio and rheological characterization, as well as texture analysis. Potential applications in transdermal delivery system were investigated by considering drug incorporation and release efficiency, antimicrobial activity, and permeation profile, which was characterized using confocal laser scanning microscope (CLSM).

2. Materials and methods

2.1. Materials

HA was kindly provided by Bioland (Cheonan, Korea). The molecular weight (M_w) of HA is approximately 0.8 MDa. HEC (average M_w = 90,000 Da), divinyl sulfone (DVS), Nile red (BioReagent, suitable for fluorescence, ⩾98.0%), sodium chloride (NaCl), sodium phosphate dibasic dodecahydrate ($Na_2HPO_4{\cdot}12H_2O$), and ILTG were purchased from Sigma Aldrich (St. Louis, MO, USA). Sodium hydroxide (NaOH, assay = 98.0%) and hydrochloric acid (HCl, assay = 35.0%) were purchased from OCI Co. Ltd. (Seoul, Korea). Sodium dihydrogen phosphate dihydrate was purchased from Junsei Chemical Co. Ltd. (Tokyo, Japan). 1,3-Butylene glycol (1,3-BG) and ethanol were used as received without further purification. *P. acnes* ATCC6919 was provided by the Korean Culture Center of Microorganisms (KCCM, Seoul, Korea). A differential reinforced clostridial agar and medium were purchased from Becton, Dickinson and Company (BD, Franklin Lakes, NJ, USA). Water was deionized and Milli-Q filtered.

2.2. Preparation of HECHA hydrogels

HECHA complex hydrogels were prepared by changing the weight ratio of HEC to HA by 100:0, 75:25, 50:50, 25:75, and 0:100 wt%, which were coded as HECHA10, HECHA31, HECHA11, HECHA13, and HECHA01 hydrogel, respectively. After measuring the weights of HEC and HA powders, they were dissolved completely in 10 mL 0.02 M NaOH solution by stirring (rpm: 250, for 1.5 h at room temperature). Then, the DVS cross-linker was added into the solution with a 1:1 molar ratio of DVS to the repeating unit of HA. The use of DVS as the hydrogel chemical cross-linker showed biocompatibility *in vitro* and *in vivo* [28]. After 12 h incubation, the cross-linked hydrogel was washed repeatedly with distilled water to remove unreacted cross-linker and residue and to neutralize the pH [29]. For further experiments, hydrogels were cut into approximately 1 cm × 1 cm × 0.5 cm (width, length, height) and then freeze-dried for 4 days by using a lyophilizer (−60 °C, 5 mmTorr, 4 days; Ilshin biobase, Seoul, Korea).

2.3. Characterization of HECHA hydrogels

The composition of HEC, HA, and dried hydrogels was characterized by FT-IR (Nexus Nicolet FTIR, Thermo Scientific, Idstein, Germany). The spectra were recorded in the range of 500–4000 cm^{-1}, and 32 scans were implemented by using a Smart Orbit ATR accessory with diamond crystal and Omnic 8.0 software.

The surface morphology of the dried hydrogel was conducted by using SEM (TESCAN VEGA3, Cranberry TWP, PA, USA) with 20 kV accelerating voltage. Cross-sections of dried hydrogel were prepared by using a sharp razor blade. The internal structure (cross-section) was sputtered with gold, then observed, and imaged.

Thermogravimetric analysis of HECHA hydrogels was carried out on a JP/DTG-60 (Shimadzu, Tokyo, Japan) in an air-conditioned environment with a flow rate of 60 mL/min, at a heating rate of 10 °C/min in the temperature range of 25–250 °C (initial sample weight: 7.4 mg). The standard material used was α-alumina powder.

2.4. Swelling ratio of HECHA hydrogels

The swelling ratio (SR) of HECHA hydrogels was measured by changing the pH at a constant temperature of 37 °C, to simulate the body temperature and application of bio-medicals. The dried hydrogel (H_d, established initial weight of hydrogel: 0.02 g) was immersed in pH solutions with different pH, which was regulated by using HCl and NaOH solutions, ranging from 1 to 13. pH of the solutions was determined by using a pH meter (Mettler Toledo, Seven Compact, Gießen, Germany). After the equilibration time (24 h), swelled hydrogels were taken out, excess solvent on the surface was gently removed, and then the swelled hydrogels (H_w) were weighed. The following Eq. (1) was used to calculate the swelling ratio of the hydrogel:

$$\mathrm{SR}\ (\%) = (H_w - H_d)/H_w \times 100, \quad (1)$$