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Hydroxyethyl cellulose hydrogel for wound dressing: Fabrication, characterization and in vitro evaluation

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ABSTRACT

In this study, new hydrogel membranes were developed based on hydroxyethyl cellulose (HEC) supplemented with tungsten oxide for further implementing in wound treatment. HEC hydrogel membranes were fabricated and crosslinked using citric acid (CA). Various tests were carried out including FTIR, XRD, porosity measurements, swelling, mechanical properties, gel fraction, and thermal gravimetric analysis to evaluate the efficiency of the prepared membranes as wound dressing material. In addition, wound healing activity of the examined membranes for human dermal fibroblast cell line was investigated employing in vitro scratching model. Furthermore, the potency of the prepared membranes to suppress wound complications was studied via determination of their anti-inflammatory and antibacterial activities exploiting MTT, ELISA, and disk agar diffusion methods. The results demonstrated that the HEC hydrogel membranes revealed an anti-inflammatory and antibacterial efficacy. Moreover, HEC improved the safety of tungsten oxide toward normal human cells (white blood cells and dermal fibroblast). Furthermore, HEC membranes loaded with WO_3 revealed the highest activities against *Salmonella* sp. pursued by *P. aeruginosa* in compared with the negative HEC hydrogel membrane. The current approach corroborated that HEC amended by tungsten oxide could be applied as a promising safe candidate for wound dressing material.

1. Introduction

Hydrogels are three-dimensional hydrophilic polymers networks, which are crosslinked via either chemical or physical bonds [1]. They have been utilized in considerable applications due to their efficiency to absorb and retain large quantities of water while keeping their mechanical and physical conformations [1, 2]. For instance, hydrogels have been implemented in various applications such as biomaterials, drug delivery systems with sustaining release of drug, cosmetics, and wound dressing agent [3–6]. Wound healing is a complicated process involves a series procedure for recovering cellular structures and epidermal tissue of damaged skin to its normal structure and function. Moreover, it is an indispensable process to block organ damage and increasingly recognized as a serious issue for public health concern [7]. Unhealthy people suffer from many complications during wound healing process such as wound infections by exposing to pathogenic microorganisms that lead to inflammation, which results in impediment of the healing [8]. For wound healing, hydrogels have been applied owing to its safety, and high capacity for absorption of physiological exudates from wounds providing them a moisture balance that enhances the wound healing. Moreover, smoothness and non-adhesive properties of hydrogels are the significant advantages that facilitate their removing without any damages to wounds [9]. The hydrogel system based on polysaccharides like starch, dextran, chitosan, carrageenan, alginate, and cellulose has received increasing attention in many applications [10–12]. Biodegradability, versatility, hydrophilicity, and commercial availability of polysaccharides render them an excellent alternative to the synthetic polymers [13, 14].

Cellulose is the most abundant polysaccharide available in nature with many attractive advantages including biodegradability, biocompatibility, non-toxicity, low cost, and good thermal/chemical stability [15–17]. Cellulose is a linear polysaccharide composed of 1,4-β-D-glucopyranosyl units [18,19]. The chains of cellulose molecules are

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tight networks, which are stabilized through strong intra- and inter-chain hydrogen bonds [20, 21]. Such structure makes cellulose insoluble in water, and thus, its profit value would be limited. Hydroxyethyl cellulose (HEC) is one of the most important commercial soluble cellulose derivatives. It can be employed in many biophysical, biotechnological and industrial applications as a stabilizer, thickener or coating, pharmaceutical, cosmetic [22, 23] because of its high biocompatibility with low toxicity and non-immunogenicity [24].

Metal oxides have been drawing much interest as antimicrobial agents for numerous applications. A significant feature of tungsten oxide over organic based antimicrobial agents is the highest melting point lead to their much greater stability [25]. Therefore, it is potential to fabricate them into the polymer networks by various processes at elevated temperatures. Different morphologies of WO_3 have been synthesized by various approaches such as one-dimensional nanorods [26], two-dimensional nanoplates [27], and spherical nanoparticles [28]. The unique properties and structures of WO_3 nanoparticles make them proper material candidates for several industrial and medical applications such as sensors [27], water treatment [26], electrochromic [29], anti-cancer [30], and antimicrobial [31]. To prompt the wound healing and tissue regeneration, effective wound dressings are boosted by antimicrobial and anti-inflammatory agents for impeding the local infection of wounds with pathogenic microorganisms and being the healing process [32]. For these reasons, we picked the WO_3 for supporting the prepared hydrogel membrane to be active wound dressing material. Although potential health impact of WO_3 is interested, the cytotoxicity hinders it to exploit in the medical applications. Accordingly, herein, we proposed the preparation of HEC hydrogel membrane loaded with WO_3. The FTIR, XRD, mechanical properties, swelling ability, gel fraction, thermal analysis of these hydrogels were examined to study the effect of WO_3 on hydrogel membrane properties. Subsequently, antibacterial activity and wound healing potentiality of the prepared hydrogels membranes to halt wound complications including excessive immune response (inflammation) and bacterial infections were investigated.

2. Materials and methods

2.1. Materials

Hydroxyethyl cellulose (Mw = 250,000), citric acid, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and lipopolysaccharides (LPS) from *Escherichia coli* were purchased from Sigma Aldrich, Germany. Dimethyl sulfoxide (DMSO) was purchased from Merck, Germany. Fetal bovine serum (FBS) was purchased from GIBCO Company (USA) while Dulbecco's Modified Eagle's Medium (DMEM) and Roswell Park Memorial Institute (RPMI) media were from Lonza, USA. Sodium chloride, peptone, yeast extract, and erythromycin antibiotic were purchased from Sigma-Aldrich (St. Louis, MO, USA). Tungsten oxide was prepared as previously described [33]. All the chemicals were of highest purity and used without further purification.

2.2. Methods

2.2.1. Hydroxyethyl cellulose hydrogel membrane preparation

HEC hydrogel membranes were prepared by the casting method. Briefly, 2.5 g HEC was added to 100 mL distilled water and stirred continuously at 60 °C for 1 h to form a homogeneous solution. Then, citric acid (0.5%) was used as a crosslinker with continues stirring for 2 h to complete the crosslinking step. After that, different concentrations of WO_3 (0.02, 0.04, and 0.08%) were mixed with the solution and stirring for 30 min to get a homogeneous solution. Finally, 15 mL of the mixtures were transferred to Petri dish and dried overnight at 50 °C. After drying, the hydrogel membranes were stored in desiccator till used.

2.3. Characterization

2.3.1. Fourier transform infrared spectroscopy (FT-IR) analysis

A Shimadzu FTIR-8400 S (Japan) Fourier transform infrared spectroscopy (FT-IR) was used to record the hydrogels IR spectra of HEC and HEC loaded with WO_3. For all spectra, thirty scans were collected with a 4 cm^{-1} resolution.

2.3.2. X-ray diffraction (XRD) analysis

X-ray diffraction analysis of pure WO_3 and hydrogels samples (HEC and HEC loaded with WO_3) were carried out using Shimadzu X-Ray diffraction (7000, USA, Cu-Kα radiation). The radiation wavelength was 1.5406 Å. The data were acquired in the form of 2θ versus intensity (a. u) chart.

2.3.3. Porosity measurements

Porosity was measured according to Yin et al. technique [34]. Hydrogels membranes samples weight were determined (W_1) after drying at 50 °C in a vacuum oven for 2 h. After that, the samples were immersed in absolute ethanol for 4 h. The swollen samples were weighed after wiping off the excessive surface ethanol with filter paper (W_2). The porosity was calculated according to the equation:

$$P\ (\%) = (W_2 - W_1)/pV \times 100\%.$$

where "P" represents porosity percentage, W_1 and W_2 represent the weight of the hydrogel before and after being immersed in absolute ethanol, respectively, "V" is the volume of the hydrogel, and "*p*" represents the density of absolute ethanol.

2.3.4. Gel fraction determination

The obtained HEC hydrogel membranes with/without WO_3 were dried in a vacuum oven at 50 °C for 24 h and weighted (W_0). Then, the membranes were soaked in distilled water at 37 °C for 24 h up to an equilibrium swelling weight (for removing the leachable or soluble parts from the membrane). Afterward, the hydrogel membranes were dried again at 50 °C in a vacuum oven and weighed (W_1). The gel fraction (GF %) was calculated by the following Eq. (1) [35].

$$\text{Gel fraction}\ (GF\%) = (W_1/W_0) \times 100 \quad (1)$$

Tests were conducted in triplicate to minimize error and were reported as a mean value.

2.3.5. Swelling behavior

The Swelling test is intended for the assessment of material capacity in absorbing solvents. Water was used as a solvent for swelling test. Hydrogel membrane samples were cut into 1.5 cm × 1.5 cm pieces, dried at 50 °C in a vacuum oven for 10 h, and the weights of the dried samples were determined (W_0). The dried Samples were immersed in Petri dishes filled with 30 mL of distilled water and incubated at 37 °C. The swollen samples were weighed at definite time intervals after wiping off the excessive surface water with wet filter paper. The water uptake of the hydrogel membrane was then calculated by the following Eq. (2) [36].

$$\text{Water uptake}\ (\%) = [\{W_t - W_0\}/W_0] \times 100 \quad (2)$$

where, (W_t) is the weight of swollen hydrogel membrane at a time (t) and (W_0) is the initial weight of samples. The experiments were conducted in triplicate to minimize error and were reported as a mean value.

2.3.6. Mechanical properties

Thicknesses of the hydrogel membranes were measured with an electronic digital micrometer (Mitutoyo, Japan), which has a sensitivity of 0.001 mm, and measurements were taken from five random