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Fabrication and characterization of ovalbumin films for wound dressing applications

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ABSTRACT

A great number of people suffer from burning injuries all around the world each year. Applying an appropriate wound dressing can promote new tissue formation, prevent losing water and inhibit invasion of infectious organisms. In this study, egg white with a long standing history, as a homemade remedy, was fabricated as a wound dressing for burn injuries. For this reason, ovalbumin films were cross-linked by 1-ethyl-3-3-dimethyl aminopropyl carbodiimide hydrochloride (EDC) with different concentrations (1, 5 and 10 mM) using three concentrations of ethanol. Physical–chemical characterizations including Fourier transform infrared spectroscopy (FTIR), gas transmission rate (GTR), tensile mechanical tests, water uptake and degradation rate were performed on the samples. The sample with 5 mM crosslinking agent at 70% ethanol was considered as the optimized one with 417 kPa of ultimate tensile strength, 64% elongation at break and 230% water uptake. In addition, biological evaluations conducted by MTT and live/dead assay indicated no sign of cyto-toxicity for all the samples. Moreover, scanning electron microscopy (SEM) showed that the fibroblast cells were well spread on the sample with the formation of filopodia. In conclusion, modified ovalbumin can be applied as the base material for fabrication of wound dressing and skin care products.

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1. Introduction

Skin, the largest organ of the human body, generally plays a critical role in the protection of wounds, prevention of infection, and homeostasis which allows us to carry out our daily activities [1–4]. In addition, it has the ability to regulate body temperature, receive external stimuli, and assists in the synthesis of vitamin D [3]. Whenever, integrity and function of a damaged tissue are lost in localized areas of injury, continuity of living tissue will be restored by healing and integration response of several cell types to the injury [1,3,5]. The natural wound healing process consists of balanced and coordinated activity of inflammatory, vascular, connective and epithelial tissues [6]. Full-thickness skin burns with more than 4 cm in diameter will not heal properly without grafting, and require replacement of both epidermal and dermal layers of skin [7,8]. A surgeon's goal is to achieve skin integration at the site of injury by using all the available medical and clinical possibilities (tools) and techniques [3]. Sometimes, during healing, the skin injury demands to be covered by a proper wound dressing. The best dressing is the one that provides a good environment for re-epithelialization in addition to infection prevention, patient pain attenuation, and appropriate cosmetic outcome [9].

No single material can provide all the requirements for all the stages of the wound healing process. Some of the commonly used biomaterials which find their ways in fabrication of wound dressings are collagen, gelatin, silk fibroin, chitin/chitosan, alginate, hyaluronic acid, chondroitin sulfate and polyglycolic acid/polylactic acid [5,7,10–12]. In recent years, a large number of researches have committed their time to produce a new and improved wound dressing by synthesizing and modifying biocompatible materials.

It is well known that proteins, such as serum albumin, play an important role in the wound healing process [13]. To create a novel biopolymer material, this study was focused on egg white which has been applied to treat wounds as a home remedy since ancient times. Egg white protein is a major raw ingredient for the food and pharmaceutical industries because of its technological, nutritional, biological, and functional properties including foaming as an emulsifying agent and gelling. Moreover, it has been also used for nonfood application such as medical use (lysozyme and ovotransferrin) [14–16].

The natural polymer films, especially protein films, have some unfavorable properties such as fragility of the material, excessive water solubility and poor water vapor permeability [17–19]. To answer issues on flexibility of the films, plasticizers are added to the structure [17,19]. The

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possibility of using glycerol as a plasticizing agent for natural protein films, like gelatin-chitosan films, has been studied frequently [17]. The albumin films like other natural ones had to be plasticized to increase the film flexibility through reduction of intermolecular forces acting along polymer chains. Therefore the film flexibility will be improved, however, its strength will be reduced against gases and vapors due to the enhancement in the segmental movement of the polymer chains [19].

Albumin films are easily dissolved in water [18,19] and should be cross-linked for enhancement of mechanical properties. In some studies, water-insoluble albumin films were prepared for application to act as a cell culture substrate by introducing intermolecular S–S crosslinking to albumin chains [18]. Moreover, 1-ethyl-3-3-dimethyl aminopropyl carbodiimide hydrochloride (EDC) as a zero-order crosslinking agent is extensively used for the formation of amide bands between amine and carboxylic acid groups [17,20]. Cross-linking of the polymers with EDC reduces in vitro degradation rate and improves mechanical properties of final product [20]. In this research, ovalbumin films were prepared, cross-linked with different concentrations of EDC and characterized as a wound dressing.

2. Materials and methods

2.1. Materials

Glycerol, EDC and ethanol were purchased from Merck, Germany. 3-[4,-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide; Thiazolyl blue (MTT), glutaraldehyde, acridine orange (AO) propidium iodide (PI), Dulbecco's Modified Eagle's Medium (DMEM), penicillin streptomycin solution, and glutamine were supplied by Sigma, USA. Chicken eggs were obtained from the farm soon after laying (female Lohman LLS Classic lineage chicken, with the age of 145–150 days). Human dermal fibroblasts (AGO 1523) were obtained from the National Cell Bank, Pasteur Institute of Iran. Fetal bovine serum (FBS) was obtained from NanoBioArray, Iran.

2.2. Film preparation

The egg white (EW) was removed from the shell and separated from the egg yolk. The EW homogenized at 3000 rpm and filtered through a 100 μm pore size filter to separate the protein solution from the foams and particles. In the next step, glycerol as plasticizer was added to the solution dropwise at the concentration of 7.5% V/V and mixed for a few minutes on the magnetic stirrer. After casting the resulting slurry on a flat square area of a poly(ethylene terephthalate) (PET) mold, the solutions were dried at 37 °C overnight under dry air circulation. A transparent film with a thickness of 0.5 mm was removed from the mold easily and stored in a dry environment at 4 °C. The mechanical properties of the EW films were improved by chemical crosslinking process. A number of 9 different samples were fabricated by immersion of films into 50%, 70% and 100% ethanol/water mixed solvent containing 1, 5 and 10 mM EDC at 4 °C for 24 h. In order to prevent the dissolution or loss of the films during the crosslinking process, ethanol was used as a solvent for this step. The reaction was completed as stated in Fig. 1. The treated films were thoroughly washed with double distilled water (for 3 days) to eliminate any excess EDC and urea byproduct. The resultant transparent thin film allows the inspection of the wound bed without the need to remove the dressing.

2.3. Characterization

2.3.1. Swelling and degradation measurement

The swelling test was performed by cutting the dried films into small pieces, weighting the samples ($W_{initial}$) and immersing them in phosphate buffer saline (PBS, pH = 7.4) at 25 °C. The films were removed from PBS at specific intervals and the wet weight (W_{wet}) of the samples determined immediately. The data were recorded until disintegration occurred in the sample which considered as degradation time. Each swelling value was averaged from three parallel experiments.

The water absorption percentage (swelling ratio) at each time point is calculated through Eq. (1)

$$\text{Swelling ratio } (\%) = \frac{W_{wet} - W_{initial}}{W_{initial}} \times 100. \quad (1)$$

Disintegrated parts were poured on a filter paper and allowed to get fully dried. Afterward, their gel yield ratio was reported through Eq. (2)

$$\text{Gel yield } (\%) = \frac{W_{initial} - W_{dry}}{W_{initial}} \times 100. \quad (2)$$

2.3.2. FTIR spectroscopy

The specimens were cut into powder and dried in vacuum oven for 24 h. The infrared spectra of the dried samples in KBr discs were recorded using a Fourier-transformed infrared spectrophotometer (Nicolet-NEXUS 670, USA). Spectra recording was performed at a resolution of 4 cm^{-1} over the wavelength range 4000 to 400 cm^{-1}. The uncross-linked protein film was used as a control.

2.3.3. SDS-polyacrylamide gel electrophoresis

The SDS-PAGE analysis was used to determine the variation in molecular weight of egg white protein before and after homogenizing process by performing the Bio-Rad instruction manual (Mini protein tetracell) and silver nitrate staining procedure.

2.3.4. Gas transmission rate (GTR)

According to the ASTM D3985 standard method, the gas transmission rate (GTR) of EW films was measured at constant temperature (25 °C) and relative humidity (0% RH) conditions. The film samples were mounted in a diffusion chamber with a transmission area of 5 cm^2. Dried air was applied to the compartment at one side of the chamber (lower half of the chamber) while compartment at the other side vacuumed. Molecules of gas permeating through the films are passed through the sensor allowing measuring the GTR as ml/s from 5 cm^2 transmission area directly.

2.3.5. Mechanical strength

The thickness of the rectangular specimens with dimensions of 40 × 10 mm^2 was measured using a digital micrometer. The mechanical properties of the samples were determined by uniaxial tensile testing machine with a 50 N load cell and an extension rate of 10 mm/min. The film strip was hold between two clamps and pulled by one clamp. The length of polymeric film bounded within clamp was 1.5 cm from both sides. The tensile strength and elongation at break were calculated according to previous published reports [21]. Mechanical tests were performed on at least 3 samples after immersing the specimens in PBS at 37 °C for 24 h.

2.4. In vitro biocompatibility

2.4.1. MTT assay

Human fibroblast cell lines (AGO 1523) were cultured in DMEM medium supplemented with FBS (10%), penicillin (100 U/ml), streptomycin (100 μg/ml) and glutamine. Culture plate was maintained at 37 °C in an incubator containing 5% CO_2. The samples with 0.5 mm thickness and 6 cm^2 surface area were sterilized under ultraviolet radiation for 30 min at each side and extracted in 1 ml culture medium at 37 °C for 1, 3 and 7 days based on ISO 10993-12. The same amount of medium without any sample was kept in the same condition as negative control. The cell viability was determined by MTT assay based on previous published reports [22]. Briefly, AGO cells were seeded in 96-well plate at a

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