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Preparation and characterization of in situ chitosan/polyethylene glycol fumarate/thymol hydrogel as an effective wound dressing

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ABSTRACT

In the present study, polyethylene glycol fumarate (PEGF) was synthesized as a component of blend solutions via polycondensation polymerization and characterized by different tests in order to determine its functional groups and its physical properties included melting and crystallization temperature, enthalpy of fusion and average molecular weights. Wound dressing films based on chitosan (Ch), PEGF and thymol (Th) were fabricated by solvent casting method with different formulations contained 80%(w/w) chitosan and 20%(w/w) PEGF as polymeric components and different amounts of thymol consisted of 0, 0.6, 1.2 and 1.8%(v/v) as pharmaceutical additives of blend solutions. These films were evaluated by different essential tests included Fourier transform infrared spectroscopy (FTIR), tensile testing, water vapor transmission rate (WVTR), water vapor uptake, equilibrium water uptake, water solubility, swelling, scanning electron microscopy (SEM) and antibacterial activity tests. The blend film contained 1.8%(v/v) thymol demonstrated optimal properties included acceptable mechanical properties, better absorption of water vapor or liquid water, higher water vapor transmission rate and air permeability, acceptable water solubility, superior swelling level, more porous structures and rough surfaces and the excellent antibacterial activity against both gram-negative and gram-positive bacteria which make it a suitable candidate for wound dressing applications.

1. Introduction

Yearly, a portion of medical expenses belong to skin damages and their related diseases. From past to present, the therapeutic approaches can be summarized in applying natural and synthetic bandages, cottons, gauzes, different types of debridement (e.g. surgical, enzymatic, biosurgical), hyperbaric oxygen therapy (HBOT), extracorporeal shake wave therapy (ESWT), laser therapy, electrical stimulation, ultrasound therapy, using topical products (e.g. lotions, ointments, gels, powders and creams) and wound dressings [1–2]. Among these, many researches have focused on different types of wound dressings due to their unique properties. This kind of treatment is considered as a safe one which can be used in combination of one or more therapeutic methods as mentioned above [1]. Today, an ideal wound dressing in form of film, foam, hydrocolloid, hydrogel, hydrofiber and etc. should be biocompatible, nontoxic and non-adhesive. Furthermore, it must protect the wound against bacteria, absorb wound exudates and toxic components, allow vital gas exchanges, support angiogenesis, preserve moisture in the wound-wound dressing interface, create thermal insulation in the wound area, have an acceptable quality, be convenient and sterilizable, be produced in different sizes in order to cover whole wound area and finally be economically affordable [1,3–6]. Among the biological (namely autografts, allografts, xenografts or made by biological materials) and synthetic wound dressings or combination of both, due to the biologic wound dressing problems included finding suitable donor, transmission of viruses, antigenic challenges, risk of infections, pain feelings, dehydration of the wound bed, scar forming at the wound margins, the fragility and difficulty of incorporating them into the wound site and finally ethical issues or synthetic wound dressing challenges such as the degradation rate, the amount of absorbency, biological issues for example toxic products of degradation, difficulty in (film, fiber, sponges and etc.) formability and so on, the combined wound dressing is preferred [7–12]. Physical or chemical modifications, graft polymerization with other natural and synthetic polymers, nano-composites and blend solutions are prevalent methods in order to fabricate combined wound dressings. Among these techniques polymer blending has revealed desirable biological, mechanical and degradability properties in comparison to utilize each components individually. In this case, the most challengeable point is the choice of appropriate materials [13–17]. One of the most attractive biopolymer with outstanding properties in wound dressing applications is chitosan [14,18]. Chitosan is a polycationic polysaccharide which is biocompatible, biodegradable, antibacterial, antitumor, antioxidant,

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anti-inflammatory, angiotensin converting enzyme inhibitor (ACE Inhibitor) and pain relief. Furthermore, it promotes cell proliferation (specially mitogenic activity on human keratinocytes and fibroblasts) and participates in all stages of wound healing process include hemostatic properties and promoting infiltration and migration of neutrophils and macrophages at early stages of wound healing then taking part in forming of granulated tissue which facilitated forming of fibrous tissue and re-epithelialization. Reduction of scar tissue and enhancement of related growth factors in wound healing process are its other benefits [13, 18–24]. Among these excellent properties of chitosan, it demonstrates some weaknesses include low mechanical strength, brittleness or fragility and insolubility in inert or alkaline environments that restrict its uses in wound dressing applications [13–16]. Another polymer which is categorized in synthetic polymer group and has attracted a lot of attention because of its amazing properties is called polyethylene glycol fumarate. PEGF is an unsaturated polyester based on polyethylene glycol (PEG) and fumaric acid which is biocompatible and biodegradable. Its steric bonds in main backbone allow PEGF to form in situ hydrogels that makes it a suitable material for wound healing applications [25–29]. PEGF macromeres can be produced via polycondensation polymerization of PEG and fumaryl chloride (FuCl) as monomers and propylene oxide (PO) as a proton scavenger and then cross-linked chemically, optically or thermally. Each cross linking agent impresses polymerization efficiency and specific properties of final products [27,30]. Despite of excellent properties of PEGF, its poor film formability restricts its application as a wound dressing. To fix the defects of chitosan and PEGF, making a blend of them is suggested so that the final material will obtain its biological and physical properties from chitosan and its mechanical properties from PEGF. At last we will have a new material with improved properties without wasting time and money for detecting totally a new material [17,25, 28]. Hashemi-Doulabi et al. [25], investigated these blend films comprised of different amounts of chitosan and PEGF as effective wound dressings. They concluded that the blend films with the ratio of 80%(w/w) chitosan to 20%(w/w) PEGF demonstrated acceptable mechanical properties, better transparency, higher water uptake, higher water vapor transmission rate and better antibacterial properties in comparison of other compounds and chitosan film as a control. This ratio also showed better miscibility in 30, 40 and 50 °C temperatures and more interaction of functional groups of chitosan and PEGF [26]. Another material which can improve the properties of such blend films is thymol. Thymol is a big part of thyme essential oil (36–55%) which has its properties however the studying of this material is easier than a multi components material such as essential oils. Botanically thymol belongs to lamiaceae family and structurally because of its phenol group, it has low solubility in water versus alcohols (e.g. ethanol) [31–33]. Thymol has exhibited some excellent properties include antioxidant, anti-inflammatory, local anesthetic, pain-relief, antiseptic, anticancer, antifungal and specially antibacterial properties against both gram-positive and gram-negative bacteria even on antibiotic resistant ones however its sensitivity on each species of microorganisms is variable [32–35]. Thymol is more compatible with human skin cells in comparison of chemical drugs (antibiotics) which make it a potent candidate in wound healing applications with the advantage of less side effects on human bodies and promoting wound healing process through a natural way [33,36]. A study by Hammer et al. [37], on 52 different plant oils and extracts (e.g. lemongrass, oregano, bay, thyme and etc.) showed that the maximum antibacterial activity of these kind of materials will occur under concentrations of 2%(v/v). Altiok et al. [38], investigated chitosan films contained different amounts of thyme oil include 0.2, 0.4, 0.6, 0.8, 1, 1.2%(v/v) as wound dressing films. They found that enhancing thyme oil content in chitosan films would slightly increase water vapor permeability, oxygen transmission rate (OTR), swelling amount and antibacterial activity while decreasing mechanical properties that was related to structural bubbles made by thyme oil. They concluded that the optimum concentration of thyme oil which is effective on wide range of microorganisms and also had an optimum properties for wound healing applications could be at 1.2%(v/v). The aim of this research is fabricating a blend film based on chitosan, PEGF and thymol by solvent casting method and investigating this film for wound dressing applications.

2. Materials and methods

2.1. Materials

Polyethylene glycol (average $M_w = 3$ kDa), toluene, fumaryl chloride (distilled at 161 °C and ambient pressure prior to use), anhydrous dichloromethane (DCM), propylene oxide, 1-vinyl-2-pyrrolidone (NVP), sodium hydroxide (NaOH), chitosan (low molecular weight, 20–300 cps, degree of deacetylation ~75–85%), ethanol, magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_2O$), tryptic soy agar (TSA) and Luria-Bertani agar (LBA) were all purchased from Merck (Germany). Thymol, acetic acid and phosphate-buffered saline tablets (PBS tablets, pH = 7.4, 0.01 M) were supplied by Sigma-Aldrich (USA), Titrachem (Iran) and Medicago® (Sweden), respectively.

2.2. Synthesis and characterization of PEGF

At the first step, 0.03 mol PEG in 6 ml DCM and 0.0299 mol of purified FuCl in 3 ml DCM were dissolved separately. Then PEG solution was poured into a 25 ml three-necked round-bottom flask which equipped with a condenser and contained PO with the molar ratio of 2:1 to the mixture so that stirring strongly. FuCl solution was added dropwise to the reaction flask through a separatory funnel. This reaction was done under nitrogen atmosphere and the temperature of −2 °C at the early stage (until all amounts of FuCl was finished) then the reaction flask was placed in an oil bath and the temperature was changed to the range of 30–35 °C by a heater equipped with a temperature sensor. This reaction was continued about 8 h. The synthesized macromeres were washed several times with NaOH 0.1 N in order to eliminate byproducts (e.g. chlorinated products) formed in the main reaction. Then the purified macromeres were dried at room temperature and placed in a desiccator for further desiccation. Eventually, final macromeres were stored in the freezer (−19 °C) until characterization and use. The purified PEGF macromeres were characterized by FTIR instrument (4000–400 cm^{-1}) (RXI, Perkin Elmer, USA). The specimens were prepared in form of KBr disks at room temperature. All spectra were recorded at the resolution of 4 cm^{-1} and 32 scans. Melting temperature (T_m), enthalpy of fusion (ΔH_m), the crystallization temperature (T_c) and the crystallinity percent of PEGF macromeres were determined by a STAR system differential scanning calorimeter (DSC) (Mettler, Toledo, Switzerland) in the range of −100 °C to 100 °C.

3 mg of PEGF macromeres were used under nitrogen atmosphere with the flowing rate of 50 ml/min. The macromeres were cooled from room temperature to −100 °C and then were heated to 100 °C; once again the cooling action was done to reach −100 °C at the heating rate of 10 °C/min. T_m and ΔH_m were calculated by the minimum point of exothermic peak unlike T_c and the integration of this area. The crystallinity percent (X) of PEGF macromeres were obtained by the equation below [39]:

$$X = \frac{\Delta H_m}{\Delta H_m^*} \times 100 \quad (1)$$

where, ΔH_m^* is the theoretical enthalpy of fusion of 100% crystalline PEG which is 49 cal/g here. The number average and the weight average molecular weights (M_n, M_w) and also polydispersity index (PDI) of PEGF macromeres were determined by a gel permeation chromatography (GPC) instrument (YL 9100, YL instrument, South Korea). Polystyrene with a given molecular weight was used as the standard calibration and tetrahydrofuran (THF) as the PEGF solvent and the mobile phase. PEGF solution with the concentration of 0.1 ml/mg was filtered by a