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Honey/PVA hybrid wound dressings with controlled release of antibiotics: Structural, physico-mechanical and in-vitro biomedical studies

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ABSTRACT

Hydrogel/honey hybrids manifest an attractive design with an exclusive therapeutic property that promotes wound healing process. The greater the concentration of honey within the formulation, the better the biomedical properties that will be achieved. However, an increase in the percentage of honey can negatively affect the physico-chemical and mechanical properties of hybrid hydrogels. The need exists, therefore, to prepare wound dressings that contain high honey density with optimal biomedical, mechanical and physicochemical properties. In this study, a simple method for the preparation of a highly concentrated honey/PVA hybrid hydrogel with borax as the crosslinking agent is reported. Comprehensive evaluations of the morphology, swelling kinetics, permeability, bio-adhesion, mechanical characteristics, cytotoxicity, antibacterial property, cell proliferation ability and their controlling release properties were conducted as a function of crosslinking density. All the borax-induced hydrogels showed acceptable biocompatibility, and the incorporation of 1% borax in the hydrogel formulation produced optimal behaviours for wound addressing applications.

1. Introduction

The key concepts of swelling property, semi-permeability, infection control and provision of wound microenvironments relevant to cell activities, are important factors affecting wound dressing performance [1,2]. Hydrogels, biocompatible hydrophilic polymers, with the intrinsic high capacity to absorb exudates from the wound surface, can provide a moist environment to the wound area [3]. Optimal wound moisture enhances the re-epithelialization process and results in fast wound healing, a factor that is accessible by hydrogels. It seems that no single material can achieve all the requirements for an ideal wound dressing.

Poly (vinyl alcohol) (PVA), as a neutral hydrogel, has been widely used in various biomedical applications due to its bio-compatibility, hydrophilic properties and biomechanical characteristics [4]. Because of its hydrogel-forming properties and capacity to provide controlled release of antibiotics, PVA has been advanced as a potential wound dressing material [5]. The permeability of PVA to small molecules, low interfacial tension, soft consistency and transparency also contribute to its promise for wound dressing [6].

Honey is another substance that should also be taken into account due to its appropriateness for wound healing as well as its antibacterial activities [7]. However, the antibacterial and biological properties of honey as well as its composition hinge on its flora source [8]. Manuka honey, which has been used in clinical practices, manifests high antibacterial activities [7,8]. Different mechanisms have been reported to explain the antibacterial activity, including hydrogen peroxide production by glucose oxidase enzyme subsequent to the interaction of honey with wound exudate, phagocytosis inhibition by antioxidant ingredients, low water activity and acidity ($3.5 < pH < 4.5$). Apart from the antibacterial effect, wound healing acceleration, inflammation reduction and smell neutralization have been attributed to honey [7,9–11].

Because of their known merits, PVA hydrogels containing honey have been widely used as wound dressing; however, high honey concentration and PVA solubility in aqueous media are known to be major drawbacks. Structural instability and poor mechanical properties were observed following the increase of honey concentration [12]. For long-term application, therefore, this hydrogel needs to be cross-linked by physical crosslinking incorporating the freeze-thaw method or chemical crosslinking processes [13–15]. Some chemical cross-linkers such as formaldehyde with PVA negatively affect biocompatibility and may put the tissue at health risk if used in wound dressing. On the other hand, it has been reported that high temperature occurring during crosslinking destroys the enzymes in honey that are responsible for the hydrogen peroxide production,

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hence reducing its antibacterial properties [7]. Thus physical crosslinking of PVA containing honey, which requires frequent cycles of heating and cooling, seems to be an ineffective method for the formation of PVA/honey hydrogel.

To enhance the physical and mechanical properties of honey/PVA hybrid hydrogel, borax can be used as a crosslinking agent [16]. Water soluble borax with the chemical formula $Na_2[B_4O_5(OH)_4]\cdot 8H_2O$ shows low acute oral toxicity (LD_{50} ranges from 2 to 5 g/kg) without skin or respiratory sensitization. The objective of the present research was to design and fabricate a wound dressing of honey/PVA hybrid hydrogel with a high concentration of honey in the presence of borax as a crosslinking agent to solve the abovementioned problems. Physical, mechanical and morphological properties of the hydrogel were investigated and preliminary in-vitro tests were performed for its initial characterization as a wound dressing or for other biomedical applications. The contribution of borax to the crosslinking of PVA and honey promoted the physicochemical and mechanical properties of the honey/PVA hybrid hydrogel containing a high concentration of honey. Subsequently, the medicinal property of this type of hybrid hydrogel was maintained over 24 h and led to excellent wound healing management via support for cell proliferation.

2. Materials and method

2.1. Materials

Poly vinyl alcohol (PVA) (Mw = 110,000; hydrolyzed >99%), sodium tetraborate (borax; assay 99%), Mueller Hinton broth for microbiology (90922), fetal bovine serum (12203C), MTT solution (TOX1), dimethyl sulfoxide (DMSO, 67-68-5), Dulbecco's Modified Eagle Medium (DMEM, D5796), penicillin-streptomycin (P4333) and phosphate buffer saline (PBS; bio-performance certified, 0.2 μm filtered) were purchased from Sigma-Aldrich (Australia). Manuka honey was obtained from the Medicinal Honey Company (Australia). All other chemicals (potassium chloride, sodium hydrogen carbonate, sodium chloride and sodium di-hydrogen phosphate) were used in analytical grade with no further purification.

2.2. Honey/PVA hybrid film preparation

PVA (6% w/w) solution was prepared by adding powder to distilled water at 70 °C with constant stirring (1000 rpm) overnight in a sealed container. Borax solution (10% w/w) was prepared by constant stirring of borax powder in water at 50 °C for 15 min. The solutions were kept sealed for foam fabrication and sterilized in autoclave for 15 min at 121 °C. For film fabrication, the temperature of the PVA solution (95 g) was reduced to room temperature and a mass of 5 g honey was slowly added to the solution. The honey and PVA solution was mixed at room temperature for about 1 h at 400 rpm. The borax solution (3, 6 and 10% w/w, respectively) was added dropwise to the honey/PVA mixture at 4000 rpm agitation over 2 h. The final solutions were moulded in petri dishes and were kept at 50 °C overnight. The honey percentage for all samples (with 0.3%, 0.6% and 1% borax as crosslinking agent) was about 80% in the dry state after fabrication.

2.3. Pseudo extracellular fluid (PECF) preparation

0.35 g NaH_2PO_4, 0.68 g NaCl, 2.5 g $NaHCO_3$ and 0.22 g KCl were dissolved in 100 mL of distilled water to prepare pseudo extracellular fluid (pH = 8.00 ± 0.05) to be used for simulating the wound environment [17]. PECF with alkaline pH (not >8.7) can properly simulate both acute and chronic wounds with a low rate of healing and excessive protein cleavage at the wound site [21].

2.4. Swelling characterization

The samples were immersed in PECF solution at 37 °C (sample mass: PECF mass = 1:200). Swelling was allowed to continue until an equilibrium swelling state was reached, where sample weight remained constant. The change in swelling ratio (SR %) as a function of time was measured by weighing samples at time intervals and using

$$SR = \left[\frac{W_t - W_0}{W_0}\right] \times 100$$

where W_t and W_0 are the weights of samples at time t and the initial time, respectively. Before a sample was weighed, the surface PECF was gently removed with filter paper. The average value of at least three measurements ± standard deviations (SD) was reported for each experiment.

2.5. Honey leakage measurement during swelling

A refractometer (Mettler Toledo, Switzerland) was used to measure the PECF refractive index, the degree to which light was bent when passing through the swelling media, during swelling. For swelling characterization of the samples, swelling medium (without sample) was shaken for 1 min in order to homogenise the PECF/honey solution before measurement. A few drops of the swelling medium were placed on the refractometer prism and then the scale was viewed through the eyepiece. The calibration curve for refractive index–honey concentration in PECF was calculated using the same procedure beforehand, while the refractive index of the PECF was used as a reference point. All measurements were performed at room temperature at least three times and were reported as mean ± SD.

2.6. Bio-adhesion analysis

The bio-adhesion strength of the honey/PVA hybrid hydrogel was measured using a texture analyser (Stable micro system, UK). The tests were performed for the samples containing 1% borax, as it was realised that samples crosslinked with 0.3 and 0.6% crosslinking agent were degradable during the swelling process, and by employing a full thickness chicken skin (prepared fresh from slaughter) as a tissue substrate. The chicken skin (6 cm diameter) was secured with Quick Fix® Supa Glue™ on the surface of a poly methyl methacrylate (PMMA) sheet and held in the measuring system (bottom) by clamps. The square samples (2 cm length) were attached to the PMMA sheets using Supa Glue™ and placed into PECF solution for known swelling times. The samples were attached to the upper cylindrical probe by a double-sided adhesive tape and careful observation was used to ensure that sample and chicken skin came into direct contact at the beginning of the measurement. Upon contact, a preload of 1 N was applied for 45 s for each measurement and a bio-adhesion test was performed at the strain rate of 1 mm s^{-1}. The adhesion test was initiated by spreading 100 μl of distilled water over an exposed skin sample and continued until complete detachment. Results were reported as the mean ± SD of three replicates.

2.7. Morphological study

Scanning electron microscopy (TESCAN, Czech) was used to study the morphology of the sample surfaces. All samples were air-dried in a fume hood prior to sputter coating with platinum.

2.8. Mechanical properties

Tensile tests were performed using a universal testing machine (Instron, USA) at the strain rate of 10 mm/min at room temperature until failure. Samples with 1% borax were kept in a sealed container