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Development and in vitro assessment of alginate bilayer films containing the olive compound hydroxytyrosol as an alternative for topical chemotherapy

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ABSTRACT

Topical chemotherapy is the application of cancer drugs directly onto the skin, which has become a standard treatment for basal cell carcinoma. Due to the promising results in the treatment of skin cancer, topical chemotherapy has recently been applied to breast cancer patients because some breast cancer tissues are only superficial. Hydroxytyrosol, a phenolic compound from olives that is present in high amounts in Hidrox® olive extract, has been shown to have a protective effect on normal cells and selective antitumor activities on cancerous cells. The aims of the present study were to develop an alginate bilayer film containing Hidrox® and to investigate its potential use as a topical chemotherapeutic agent. Alginate films were characterized for swelling and for physical, thermal, rheological, and mechanical properties. Drug content uniformity and *in vitro* drug release tests were also investigated. The alginate bilayer films containing Hidrox®, HB2, showed controlled release of hydroxytyrosol at a flux of $0.094 \pm 0.009\,mg/cm^2/h$. The results of the cytotoxic assay showed that the HB2 films were dose-dependent and could significantly reduce the growth of breast cancer cells (MCF-7) at 150 μg/mL for a cell viability of $29.34 \pm 4.64\%$. In conclusion, an alginate bilayer film containing Hidrox® can be a potential alternative for topical chemotherapeutic agent for skin and breast cancer treatment.

1. Introduction

Topical chemotherapy refers to the application of cancer drugs directly to the skin. The Food and Drug and Administration (FDA) has approved two creams for basal cell carcinoma: 5-Fluorouracil and Imiquimod creams in 1970 and 2004, respectively. Studies have shown that anticancer creams reduce systemic side effects associated with conventional chemotherapy and can potentially allow for avoidance of invasive surgical procedures (Desai et al., 2012; Gross et al., 2007; McGillis and Fein, 2004). Patients self-treat at home, but repeated application leads to adverse side effects such as erythema and superficial erosions at affected sites (Desai et al., 2012). Some patients even experience pruritus and irritation and therefore require close follow-up observations during the course of treatment to monitor the response to the medication. Due to the promising results in skin cancer patients, topical chemotherapy has recently been applied to breast cancer (Kohrt, 2012; Lazzeroni et al., 2012). Because the breast is not a deeply seated organ, drug permeation from the skin of the breast into the target area appears to be a promising route for the delivery of anticancer drugs. Thus, the use of topical chemotherapy for breast cancer can offer an effective and safe alternative to radiation, surgery and conventional chemotherapy.

Hidrox® is a patented freeze-dried formulation of olive polyphenols obtained from the juice of fresh olives (*Olea europaea*) that was first introduced to the market by CreAgri. Hidrox® contains the antioxidant molecule hydroxytyrosol, which has an antioxidant activity (Oxygen Radical Absorbance Capacity (ORAC)) of 27,000 μmole TE/g, which is 13 times higher than ascorbic acid (ORAC 2100 μmole TE/g) (Creagri, 2010). Hydroxytyrosol or 4-(2-hydroxyethyl)-1,2-benzenediol, a naturally occurring phenolic antioxidant molecule found in olives, has been reported to exert several biological and pharmacological activities (Odiatou et al., 2013; Owen et al., 2000; Silva et al., 2015). Previous studies have demonstrated that 4-hydroxytyrosol has dose-dependent anticancer activity *in vitro* and is capable of inhibiting proliferation and inducing apoptosis in a human melanoma cell line (M14), MCF-7 human breast cancer cells, and human myeloid leukaemia HL60 cells (D'angelo et al., 2005; Han et al., 2009; Ragione et al., 2000). In addition, hydroxytyrosol was shown to reduce the

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reactive oxygen species (ROS) in normal breast cells and to protect against DNA damage (Warleta et al., 2011). Therefore, hydroxytyrosol may represent a potential alternative anticancer compound to conventional cytotoxic drugs.

Hydrocolloid films are ideal for topical drug delivery because unlike creams and gels, the matrix contains a fixed dose loaded onto a defined area and has a long residence time. In addition, the film is thin and flexible and can be trimmed into desirable sizes. A bilayer alginate-based hydrocolloid film, developed by Thu et al. (2012), consists of two layers, of which the bottom layer is drug-free while the upper layer is loaded with a drug. The drug-free layer acts to control the drug release from the upper layer, thus reducing excessive drug accumulation on the surface of the skin. Previous studies have also shown that alginate films can act as a topically controlled drug delivery system (Dong et al., 2006; Thu et al., 2012).

Hidrox® 6% (Hidrox-6), a commercial olive powder produced by Creagri, was used as the active ingredient in the present study. Hidrox-6 contains ~3% 4-hydroxytyrosol and 10% polyphenol compounds. Creagri has ascertained that hydroxytyrosol exerts the highest anti-inflammatory activity among the bioactive nutrients contained in the fruit of the olive (CreAgri, 2013). In the present study, a bilayer film containing Hidrox-6 was developed. Polymers used for film development were alginate and gelatin, while glycerol and propylene glycol were used as film plasticisers (Thu et al., 2012). Drug-free bilayer films and Hidrox-6 loaded bilayer films were first characterized for their physical, mechanical, thermal, rheological and hydration properties. Then, the bilayer films containing Hidrox-6 were assessed for uniformity of drug content and drug permeation by Franz-type cell studies. Finally, cell proliferation in a cell culture assay was performed to investigate the cytotoxicity efficacy.

2. Materials and methods

2.1. Materials

Hidrox-6 was prepared by freeze–drying the juice of organic California-grown olives and was a gift from the manufacturer, Creagri, Inc. (California, USA). Sodium alginate and gelatin powder made from bovine skin (Type B), propylene glycol, glycerol, and phosphate buffer saline (PBS) tablets (pH 7.4) were purchased from Sigma–Aldrich (USA). For in vitro cell culture studies, the MCF-7 cell line was obtained from the American Type Culture Collection (ATCC, USA). Chemicals used for cell culture included Gibco® media powder, Dulbecco's Modified Eagle's Medium (DMEM) (Invitrogen, USA), Foetal Bovine Serum (Biowest, South America), Gibco® Penicillin/Streptomycin 100X Antibiotic (Invitrogen, US), Sodium hydrogen bicarbonate ($NaHCO_3$) (Bendosen), and Trypsin-EDTA Solution 10X (Sigma–Aldrich, UK). Chemicals that were used in the bioassays include dimethyl sulphoxide (DMSO) (C_2H_6SO) (Merck, Germany) and 3-(4,5-dimethythiazol-2-yl)-2.5-diphenyl-tetrazolium bromide MTT reagent powder (Bio Basic, Canada). Tryphan blue stain 0.4% (Gibco®, USA) was used in cell counting.

2.2. Alginate film preparation

The alginate films were prepared with a solvent casting method (Boateng et al., 2008) modified from Thu et al. (2012). Two grams of gelatin powder was hydrated with distilled water for at least 10 min. The gelatin mixture was heated to 60 ± 2 °C with continuous stirring until all of the gelatin granules were dissolved. Six grams of sodium alginate was then stirred into the gelatin solution. Heat was removed once a uniform gel was obtained. Propylene glycol or glycerol was added to the warm gel with continuous stirring. The alginate gel solution was covered with parafilm and sonicated in an ultrasonicator (Branson Ultrasonicator, USA) for 1 h to remove any trapped air bubbles. For preparation of single layer films, 25 g of the gel was poured into a plastic petri dish with an internal diameter of 8.4 cm. These were dried in an oven (Membert Oven, UK) at a temperature of 30 ± 2 °C for 24 h. Dried silica gel beads were placed in the oven to maintain the RH at 54 ± 2%. The dried alginate films were stored in desiccators with silica gel beads at room temperature of 27 ± 2 °C and RH of 54 ± 2%.

For films that contained 5% Hidrox-6, 5 g of Hidrox-6 powder was added to the alginate hydrogel made with 60 mL of distilled water. To incorporate the Hidrox-6, 5 g of powder was first weighed and dissolved in 36 mL of distilled water using a magnetic stirrer. Once the Hidrox-6 dissolved to form a uniform solution, it was added to the alginate hydrogel and stirred until a homogenous gel was formed. The Hidrox-6 hydrogel was covered with parafilm and sonicated to remove air bubbles.

For bilayer films, the blank lower layers were prepared according to the method used in single layer films. The blank lower layer was made by casting 35 g of blank alginate hydrogel onto the petri dish and dried in the oven for 24 h at 30 ± 2 °C together with silica gel beads to maintain the RH at 54 ± 2%. The obtained dried blank films were approximately 4.5 ± 0.2 g. Then, 25 g of blank or Hidrox-6 hydrogel was poured on top of the dried blank films. The films were then quickly placed in the oven with the temperature set at 40 ± 2 °C for 6 h. Silica gel beads were used to control the drying process. The dried bilayer films were stored in desiccators with silica gel at room temperature of 27 ± 2 °C and RH of 54 ± 2% until further evaluation.

Table 1
Composition of sodium alginate film formulations.

Formulation	Constituents					
	Sodium alginate (% w/v)	Gelatin (%w/v)	Glycerol (% v/v)	Propylene glycol (% v/v)	Distilled water (% v/v)	Hidrox-6 (% w/v)
Single layer films						
S1 (blank)	6	2	–	–	100	–
S2 (blank)	6	2	2	2	96	–
S3 (blank)	6	2	–	2	95	–
S4 (blank)	6	2	2	–	95	–
HS2	6	2	2	2	96	5
Bilayer films						
HB2 (upper layer)	6	2	2	2	96	5
(lower layer)	6	2	2	2	96	–
B2 (upper layer)	6	2	2	2	96	–
(lower layer)	6	2	2	2	96	–