Materials Science and Engineering C 71 (2017) 498–503

Contents lists available at ScienceDirect

Materials Science and Engineering C

journal homepage: www.elsevier.com/locate/msec

Biophysical and biological characterization of intraoral multilayer membranes as potential carriers: A new drug delivery system for dentistry

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ARTICLE INFO

Article history:
Received 26 August 2016
Received in revised form 3 October 2016
Accepted 16 October 2016
Available online 17 October 2016

Keywords:
Layer-by-layer coatings
Alginate
Chitosan
Cell viability
Chlorhexidine
Controlled drug release

ABSTRACT

The current study developed through layer-by-layer deposition a multilayer membrane for intraoral drug delivery and analyzed the biochemical, functional, and biological properties of this membrane. For that purpose, we designed a three-layer chlorhexidine-incorporated membrane composed by pure chitosan and alginate. The biochemical, functional, and biological properties were analyzed by the following tests: degradation in saliva medium; controlled drug release; water absorption, mass loss; pH analysis; and biocompatibility through fibroblast cell viability by MTT assay. All tests were conducted at three different periods (24, 48 and 72 hours). The results demonstrated that hybrid membranes composed by alginate and chitosan with glycerol had greater water absorption and mass loss in buffer solution and in artificial saliva. The controlled drug release test revealed that the hybrid membrane exhibited greater drug release (0.075%). All chlorhexidine-incorporated membranes reduced the cell viability, and chitosan membranes with and without glycerol did not interfere with fibroblast viability. The biochemical and biophysical characteristics of the designed membranes and the findings of cell viability tests indicate great potential for application in Dentistry.

1. Introduction

The research, development, and increasing health application of biomaterials encourage the search for new bioactive, biodegradable, non-toxic, and easy-handling polymers with different applications [1]. Biomaterials are those used for tissue replacement, whose biophysical and chemical properties should stimulate the necessary response of living tissues [2–3].

Chitosan is a natural polymer with hemostatic, fungicidal, and antibacterial properties that stimulates cell migration and proliferation, reorganizing the cell histoarchitecture [4–6]. This polymer raised the interest of different health specialties as pharmacy, medicine, and dentistry due to the characteristics of biodegradability, biocompatibility, and non-toxicity [7–9]. Because of these biological characteristics, chitosan has promisingly properties for use in tissue engineering as scaffolds [10].

Alginate is another relatively inert, biocompatible, and biodegradable polymer that forms gel matrices with high porosity and good mucoadhesive properties [5,11–12]. Alginate activates the macrophages, triggering an inflammatory response in the organism and initiating the wound healing process.

Over the years, the researchers seek a therapeutic resource to be applied in mouth, adhering to the oral mucosa, to treat surgical wounds, wounds caused by physical and/or chemical trauma, and other types of ulcerated oral lesions [13–14]. This is particularly significant in the treatment of children undergoing oral trauma, whose non-compliance behavior makes difficult to apply dressings and/or topical drugs.

The development of new release systems, as the mucoadhesive systems, may enable the incorporation of drugs into these biomaterials. Mucoadhesive systems remain in close contact with the target tissue, namely the mucosa, releasing the drug at the site of action, consequently increasing the bioavailability, possibly promoting local and systemic effects. Mucoadhesion is currently explained by six theories: electronics, adsorption, wettability, diffusion, fracture, and mechanics [15].

This study developed and analyzed the biochemical and functional properties of chitosan and alginate membranes, with or without chlorhexidine.

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http://dx.doi.org/10.1016/j.msec.2016.10.032
0928-4931/

2. Material and methods

2.1. Preparation of gels

The chitosan gel was prepared by mixing chitosan (2%) (Sigma-Aldrich, St. Louis USA) into 100 mL of 2% acetic acid solution (v/v). The sodium alginate gel was prepared by mixing 5 g of sodium alginate (Sigma-Aldrich, St. Louis USA) in 100 mL of distilled water. Both solutions were stirred for 24 h and then filtered. Then, 50 g of gel were separated for addition of 1.25 g of glycerol.

2.2. Preparation of chitosan membranes

Three grams of chitosan gel were weighed in Petri dishes (6.5-cm diameter) on an analytical scale. The prepared plates were placed in an oven at 30 °C during 24 h for drying of membranes and further use.

2.3. Preparation of hybrid chitosan/alginate/chitosan membranes

The multilayer membranes were prepared with equal parts containing 1.5 g of chitosan, 1.5 g of alginate, 1.5 g of chitosan. Gels with and without glycerol were used. The layers of each material were placed individually and dried inside an oven at 30 °C for 15 min. After that, the three-layer hybrid membrane was placed in an oven for 24 h for final drying (Fig. 1).

2.4. Incorporation of chlorhexidine

Chlorhexidine (20% chlorhexidine digluconate, EC 1907/2006, Evonik Industries) was added to the gels prepared as described in item 2.1, at a concentration of 1% (m/m).

2.5. Controlled chlorhexidine release testing

In vitro release tests were performed for semi-quantitative evaluation of the release of chlorhexidine digluconate in phosphate buffer solution (PBS), pH 7.4 ± 0.2, according to the methodology of AKAKI (2005) with some modifications. The chlorhexidine-incorporated membranes were placed in Falcon tubes and 14 mL of PBS were added to cover the membrane entirely. Samples of 1.0 mL were obtained after 1, 2, 3, 4, and 5 days. After that, 1.0 mL of PBS buffer solution was added in each Falcon tube to maintain the volume of 14 mL. The absorbance of each sample was read at 255 nm using a spectrophotometer (Beckman DU-640, USA), and the values were recorded to calculate the concentration of samples. Due to the addition of 1.0 mL of PBS solution in the Falcon tubes, a correction factor was used to adjust the determined concentration.

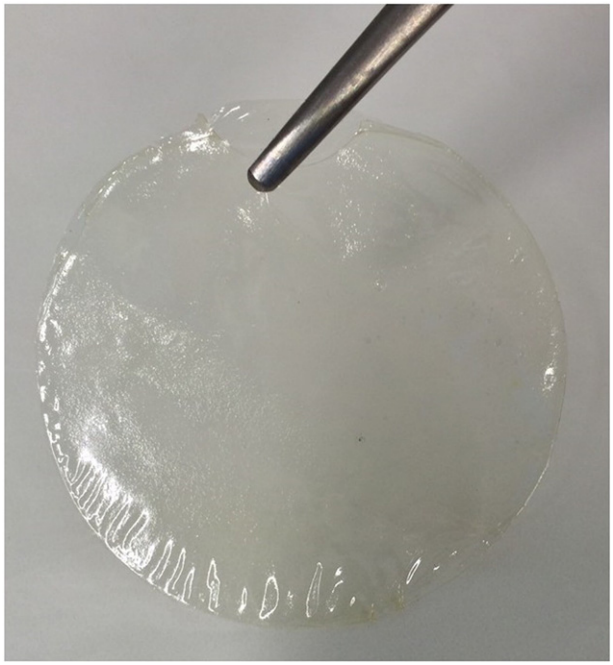

Fig. 1. Final aspect of tested membranes.

2.6. Absorption and mass loss testing

Testing of absorption and mass loss were performed in phosphate buffer saline solution (PBS) and in artificial saliva solution, which was prepared in the institution's biochemistry laboratory based on the formulation describe by Hahnel et al., 2010 [16]. The membranes were dried for 24 h at 40 °C, weighed, and immersed in 10 mL of artificial saliva solution pH 6.8 and in PBS buffer solution at pH 7.4 ± 0.02. The flasks with samples were placed in water bath at 37 °C. Membranes in artificial saliva solution were removed at determined periods of 1, 2, and 3 days. The membranes were removed at their respective periods, placed between sheets of tracing paper to remove the excess artificial saliva solution, then weighed, and the respective values were recorded. After weighing, the membranes were placed in an oven for 24 h at 37 °C, and once again weighed. Data recorded during testing were used to calculate the absorption of saliva solution and mass loss of the membranes, using standard equations for these tests.

Uptake was determined by increasing the initial membrane mass (mi). After incubation, the solution excess was removed with paper filter, and the mass (mw) was determined using an analytical balance. Then, membranes were dried at 40 °C until reaching a constant mass, which was determined (mf) to obtain the fiber mass loss. Absorbed water and mass loss are demonstrated in Eqs. (1) and (2).

$$\text{Absorbed water} = [(\text{mw} - \text{mi})/\text{mi}] \times 100 \quad (1)$$

$$\text{Mass loss} = [(\text{mi} - \text{mf})/\text{mi}] \times 100 \quad (2)$$

2.7. Cell viability testing

These tests employed NIH3T3 fibroblasts (ATCC) cultured in Dulbecco's modified Eagle's medium (DMEM) (Gibco Co, Grand Island, USA), supplemented with 10% fetal bovine serum (FBS) (Gibco Co, Grand Island, USA). The cells were kept at 37 °C in an environment of 5% CO_2 and 95% air.

The membrane extracts were prepared in DMEM culture medium without FBS, at a concentration of 0.1 g/10 mL (material/DMEM culture medium), during 24 h, at 37 °C, in an environment of 5% CO_2 (adapted from ISO 10993-12). The ISO 10993-12 advocates a proportion of 0.1 g/1 mL; however, this adaptation was made due to the need to solubilize some membranes that absorbed almost the entire content of DMEM. The groups of each membrane were defined as follows: G1 – hybrid membrane + glycerol + chlorhexidine; G2 – hybrid membrane + chlorhexidine; G3 – chitosan membrane + glycerol + chlorhexidine; G4 – chitosan membrane + chlorhexidine; G5 – hybrid membrane + glycerol; G6 – hybrid membrane; G7 – chitosan membrane + glycerol; G8 – chitosan membrane; C+ – positive control (DMEM medium with 10% FBS) and C− – negative control (DMEM medium with 1% FBS).

The cell viability test (MTT) was made by cell plating in 96-well plates (1×10^3 cells/well). After 6 h of cell adhesion, the culture medium was changed to DMEM medium with 5% FBS, down-regulating cell proliferation. The plates were incubated in humid ovens at 37 °C with 5% CO_2 for 24 h. Thereafter, the medium was changed by the different groups containing the extracts obtained from each of the membranes and positive and negative controls. The analyses were performed after