PREPARATION AND CHARACTERIZATION OF CHITOSAN/REGENERATED SILK FIBROIN (CS/RSF) FILMS AS A BIOMATERIAL FOR CONTACT LENSES-BASED OPHTHALMIC DRUG DELIVERY SYSTEM

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ABSTRACT

Objective: The aim of this study was to develop chitosan/regenerated silk fibroin (CS/RSF) films as a biomaterial for contact lenses-based ophthalmic drug delivery system.

Methods: CS/RSF films were prepared with polyethylene glycol 400 as a plasticizer by using a film casting technique. Their physicochemical properties were investigated by measuring various properties such as thickness, morphology, chemical interaction, light transparency, mechanical properties, water content, oxygen permeability, thermal properties and enzyme degradation. In addition, cytotoxicity was also studied.

Results: At optimal preparation conditions, CS/RSF films showed smooth surfaces with highly visible light transparency of >90%, which meet the visual requirement. CS/RSF films showed high water content, 59-65% by weight, and their Young’s modulus and elongation at break was in the range of 3.8-6 N/mm\textsuperscript{2} and 11.3-13%, respectively. The CS/RSF films also could be sterilized by autoclave method as they possessed high thermal decomposition temperature of >260 °C which can be confirmed by both differential scanning calorimetry and thermogravimetric analysis. In addition, CS/RSF films showed no degradation in stimulated tear fluid containing lysozyme for 7 d and showed no cytotoxicity by MTT assay.

Conclusion: CS/RSF films showed excellent physicochemical properties and non-cytotoxicity indicating their promising potential use as a biomaterial for contact lenses-based ophthalmic drug delivery system.

Keywords: Chitosan, Regenerated silk fibroin, Films, Contact lenses, Ophthalmic drug delivery system, Cytotoxicity

To overcome the limitation of synthetic polymer-based contact lenses, we have proposed natural polymers as a potential biomaterial for contact lenses. This is due to their advantages of non-toxicity, good biocompatibility, low inflammatory, high oxygen permeability, high optical transparency, high wettability, and good chemical and mechanical stabilities that could meet the required properties of daily disposable contact lenses. Furthermore, they can be used as daily disposable therapeutic contact lenses [26-39]. Chitosan (CS) and regenerated silk fibroin (RSF) are natural polymers of interest. CS is a natural polycationic linear polysaccharide derived from the deacetylation of chitin [40]. RSF, which is derived from degumming of the Bombyx mori cocoons and dissolution of silk fibroin respectively, is a protein mainly comprised of amino acids glycine, alanine, and serine [41, 42]. CS films showed good flexibility, high light transparency and high water content but it is highly sensitive to lysozyme degradation [32-35, 43]. RSF films offer the advantages of high oxygen permeability, non-toxicity, excellent biocompatibility, and also excellent wound healing properties, but it is quite brittle [26-31]. Therefore, the blending of CS and RSF is a possible solution to improve the properties of films for creating the materials of daily disposable therapeutic contact lenses. Moreover, the blending of CS with RSF shows good compatibility between two different materials by hydrogen bonding interaction [36, 40].

INTRODUCTION

Topical drug administration is a common approach to treat ocular disorders. Currently, more than 90% of marketed topical eye drops are in the form of solutions and suspensions because of their convenience and ease of administration [1-3]. However, eye drops are notorious for poor ocular bioavailability with less than 5% of administered drugs entering the anterior chamber and reaching the intraocular tissues [2, 4-6]. A large part of the drugs is lost into the systemic circulation by blinking, rapid tear turnover rate and drainage into the nasal cavity. To maintain sustained therapeutic drug levels, frequent administration or large doses are often required. Consequently, this reduces patient compliance, increases local side effects, and also results in pronounced systemic exposure [2, 7-9]. Recently, daily disposable contact lenses-based ophthalmic drug delivery systems have been proposed as alternative ophthalmic drug delivery systems for increased ocular bioavailability [10-14]. These approaches can be administered without any surgery and have been demonstrated to produce sustained drug release for a prolonged period by increasing the residence time of the drug on the ocular surface [12, 15-17]. The benefit of daily disposable therapeutic contact lenses would be the delivery of the correct medication dosage at an approximately constant rate, thereby eliminating the frequent application of topical eye drops and, more importantly, leading to more benefits to the patient with substantially increased efficacy. Typically, commercial daily disposable contact lenses are usually made from synthetic polymers, such as poly (2-hydroxyethyl methacrylate) (pHEMA)-based hydrogel and silicone based-hydrogel (SiH). pHEMA-based hydrogel contact lenses provide high water content of up to 80% and softness that promotes comfortable wearing. Their main disadvantages are low strength [18-21]. SiH contact lenses, in contrast, have high oxygen permeability and low adhesion to the bacteria, but they are low in water content with a hydration ability of <45% [20-25].

To overcome the limitation of synthetic polymer-based contact lenses, we have proposed natural polymers as a potential biomaterial for contact lenses. This is due to their advantages of non-toxicity, good biocompatibility, low inflammatory, high oxygen permeability, high optical transparency, high wettability, and good chemical and mechanical stabilities that could meet the required properties of daily disposable contact lenses. Furthermore, they can be used as daily disposable therapeutic contact lenses [26-39]. Chitosan (CS) and regenerated silk fibroin (RSF) are natural polymers of interest. CS is a natural polycationic linear polysaccharide derived from the deacetylation of chitin [40]. RSF, which is derived from degumming of the Bombyx mori cocoons and dissolution of silk fibroin respectively, is a protein mainly comprised of amino acids glycine, alanine, and serine [41, 42]. CS films showed good flexibility, high light transparency and high water content but it is highly sensitive to lysozyme degradation [32-35, 43]. RSF films offer the advantages of high oxygen permeability, non-toxicity, excellent biocompatibility, and also excellent wound healing properties, but it is quite brittle [26-31]. Therefore, the blending of CS and RSF is a possible solution to improve the properties of films for creating the materials of daily disposable therapeutic contact lenses. Moreover, the blending of CS with RSF shows good compatibility between two different materials by hydrogen bonding interaction [36, 40].

Nowadays, there are very few reports on therapeutic contact lenses using a combination of natural polymers [35, 40], especially; there are no reports on the combination of CS/RSF for contact lenses. Therefore, the purpose of this study was to develop CS/RSF films as the biomaterials for contact lenses-based ophthalmic drug delivery system. The physicochemical properties of CS/RSF films were investigated by measuring various properties such as thickness, morphology, chemical interaction, light transparency, mechanical properties, water content, oxygen permeability, thermal properties and enzyme degradation. In addition, cytotoxicity was also studied.
Preparation of RSF

RSF was prepared according to Yamada et al. (2001) and Ajiwawa (1998) [44, 45]. Briefly, raw silk yarns of Bombyx mori were degummed twice by boiling in a 0.5% (w/v) sodium carbonate (1998) [44, 45]. Briefly, raw silk yarns of Bombyx mori were degummed twice by boiling in a 0.5% (w/v) sodium carbonate (1998) [44, 45].

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CS/RSF films were prepared by a casting method [37]. Briefly, 2% (w/v) RSF aqueous solution, 2% (w/v) of CS solution, dissolved in CS/RSF films were prepared by a casting method [37]. Briefly, 2%

Thermal properties

Differential scanning calorimetry (DSC) was performed to determine the thermal properties of the CS/RSF films using DSC 3-STAR System (Mettler Toledo (Thailand), Bangkok, Thailand). The thermal decomposition temperature of each sample was examined under a nitrogen atmosphere with a flow rate of 50 ml/min [36]. Thermogravimetric analysis (TGA) was performed using TGA/DSC 3-STAR System from -20 °C to 400 °C at a heating rate of 20 °C/min under a nitrogen atmosphere with a flow rate of 50 ml/min, in a temperature range of 30-600 °C and at a heating rate of 20 °C/min [36].

Ion permeability

The ion permeability was determined using a homemade horizontal diffusion cell with an aperture of 35 mm, at 34±1 °C. The receiving chamber was filled with 35 ml of deionized (DI) water. After soaking in DI water for 24 h, a hydrated film, 0.09 mm in thickness, was placed between the two compartments of the diffusion cell and then the donor chamber was filled with 18 ml of 154 mmol NaCl solution. The conductivity of the solution in the receiving chamber was measured at time intervals by a conduct meter (Model 712 Conductometer, Metrohm PLUS, London, UK) with a load cell of 5 kg, a function of time. Then, the apparent ion permeability was calculated using a slope (F) at steady state (dc/dt) following Fick’s law as shown in the following equation (2) [47, 50].

$$\text{Apparent ion permeability (mm}^2/\text{min}) = \left( \frac{V}{A} \right) \left( \frac{C_i}{C_r} \right) \left( \frac{dC}{dt} \right)$$  \quad (2)

Where V is the volume of the receiving chamber, A is the area of the tested film, C_i is the initial NaCl concentration in the donor, and T is the film thickness.

Oxygen permeability

CS/RSF contact lenses for oxygen permeability testing were prepared by spinning casting method in an oven at 40 °C until completely dried. The oxygen permeability of hydrated CS/RSF contact lenses, 0.2 mm of center thickness, were measured according to ISO 18369-4 (35 °C and >98% relative humidity) using the polarographic amplifier (Model 201T Permeometer, Createch/Rehder development. CO., Indiana, USA).

Preparation of RSF

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In vitro enzymatic degradation

The degradation of CS/RSF films was analyzed following their incubation in 34±1 °C in simulated tear fluid (STF) containing lysozyme 1 mg/ml (pH 7.4). The compositions of STF were sodium chloride 0.67 g, sodium bicarbonate 0.2 g, calcium chloride-2H2O 0.008 g, and deionized water added to 100 g. The film (2×2 cm²) after autoclaving and drying were weighed (initial weight, W₀). The films were immersed in 2 ml of STF containing lysozyme for 2, 5, 7, and 14 d. After that, the films were dried at 60 °C overnight and weighed after degradation (W₁). The percentage of the remaining weight was calculated as shown in the following equation [3] [33, 51-53].

\[ \text{Remaining weight} = \frac{W₀}{W₁} \times 100 \quad (3) \]

Cytotoxicity study

CS/RSF films cytotoxicity was determined by telomerase-immortalized human corneal epithelial cells line (HCECs) viability. The HCECs were seeded onto 96-well plates at 1.5×10⁴ cells per well in 100 µl of cell culture medium and incubated at 37 °C, 5% CO₂ until reaching ~90% of cell confluence (3 d). The RSF/CS films, after soaking with PBS for 24 h, were cut into the same size of 96-well plates and were then autoclaved. Then, the films were placed gently on HCECs in 96-well plates and incubated for up to 2 h at 37 °C, 5% CO₂. After 24 h, the films were carefully removed from the wells. Then, the cells were washed with PBS twice, and 100 µl of 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) solution (0.5 mg MTT/ml of medium) was added. After a 2 h reaction time, MTT formazan was extracted with dimethyl sulfoxide (DMSO) for 10 min and the absorbance of the extract was measured at 595 nm with a microplate reader (Synergy™ H1, BioTek Instruments, Inc., Vermont, USA) [54]. All results were expressed as relative viability compared to cells grown in the absence of a film (control). The ratio (%) of MTT formazan absorbance for each sample to the absorbance of MTT formazan for control represented cell viability using the following formula (4).

\[ \text{Cell viability} = \frac{\text{Absorbance of sample}}{\text{Absorbance of control}} \times 100 \quad (4) \]

Statistical analysis

The results were expressed as mean±standard deviation (SD). For all comparisons, statistical significant differences were analyzed with paired t-test or one-way ANOVA followed by Tukey’s post hoc test, and P<0.05 was considered statistically significant.

RESULTS AND DISCUSSION

The PEG400 content, 25% by weight of polymer content, and the 15 min NaOH treatment were selected based on the preliminary study. In the preliminary study, CS/RSF films prepared with PEG400 as a plasticizer were successfully developed. However, without NaOH treatment, they were extremely weak and brittle. On the other hand, with 15 min NaOH treatment, CS/RSF films showed high strength with homogeneous films. This is due to NaOH allowing new hydrogen bond formation which caused larger anhydrous crystal size and more compact structure in the films [55]. In addition, the film prepared with PEG400 at 25% by weight of polymer content showed high oxygen permeability. Therefore, all the prepared films were composed of PEG400 25% w/w with 15 min NaOH treatment. Moreover, the blended films were prepared covering the whole range of CS/RSF weight ratio of 100/0 to 0/100 (w/w).

Appearance and morphology of CS/RSF films

The prepared CS/RSF films at ratios of 100/0, 90/10, 80/20 and 70/30 (w/w) were uniformly light transparent, non-brittle and were strong enough to handle without deformation. Typically, commercial contact lenses have thickness of 0.05-0.2 mm [20, 57]. All prepared CS/RSF films showed no significant difference in thickness of 0.09±0.01 mm, indicating they were suitable for contact lenses. SEM micrographs of top surface of all CS/RSF films showed smooth surfaces without phase separation. Moreover, their cross-sections exhibited homogenous blending between CS and RSF without obvious phase disengagement as shown in table 1.

FTIR

FTIR spectra of RSF film, CS film, PEG400, and CS/RSF films, were shown in fig. 1. The RSF film without PEG400 prepared with acidic condition showed silk II conformation as evident from the peak absorption bands at 1620 cm⁻¹ (amide I, C=O stretching) and 1514 cm⁻¹ (amide II, N-H bending and C-H stretching) corresponding to the β-sheet conformation indicating crystalline portion [58, 59]. Amide III absorption band (C-N stretching and C-O stretching) was observed at 1231 cm⁻¹, silk I conformation (random coil), indicating amorphous region [58, 60, 61]. The low water solubility of the RSF film indicated the predominance of silk II structure. This was in agreement with Kweon et al. [2001] who verified that a more stable RSF structure could be induced by acetic acid [62].

The CS film without PEG400 exhibited absorption band at 1634 cm⁻¹ (C=O stretching), 1537 cm⁻¹ (N-H stretching), 1256 cm⁻¹ (O-H bending), 1065 cm⁻¹ and 1020 cm⁻¹ (C-O stretching) [36, 46, 62]. In addition, PEG400 showed characteristic absorption band at 3454 cm⁻¹ (O-H stretching), 2866 cm⁻¹ (C-H stretching) and 1095 (C-O-C stretching) [63]. Interestingly, 100CS/0RSF film showed NH₂ peak shift from 1537 to 1541 cm⁻¹ and O-H peak shift from 1256 to 1252 cm⁻¹ suggesting H-bonding between-ΟΗ of PEG400 with NH₂ or OH of CS.

The FTIR analysis of CS/RSF blended films suggested intermolecular H-bonding between CS and RSF indicating a good compatibility between CS and RSF. For example, the FTIR spectra of 70CS/30RSF film revealed the NH₂ peak of CS was shifted from 1541 to 1531 cm⁻¹ and C=O peak of RSF was shifted from 1620 to 1622 cm⁻¹. The good compatibility of the blended films was in agreement with SEM micrographs (table 1) confirming a homogeneous morphology without phase separation of the blended films.

Light transparency of CS/RSF films

The light transparency of blended films is an important property of contact lenses. The optical transmittance spectra in the range 280-780 nm of CS/RSF films were displayed in fig. 2, while the mean light transparency of each spectral range was shown in table 2. Typically, the visible light transparency (380-780 nm) of contact lenses should be more than 90% [56]. All CS/RSF films showed excellent visible light transparency of >90%, which meet the visual requirement, indicating good compatibility of the blended film. According to the American National Standards Institute of Z80.20 standard, contact lenses shall satisfy Class II UV blocking, which transmittance for UV-B (280-315 nm) and UV-A (316-380 nm) less than 5% and 30%, respectively. Although UV-B and UV-A transmittance of all prepared CS/RSF films did not meet the Class II UV blocking standard. However, all films showed significant protection against UV-B and UV-A respectively when increasing the RSF content.

100CS/0RSF film showed UV-B and UV-A transmittance of 27% and 58% respectively while 70CS/30RSF was reduced to 12% and 50%, respectively. In addition, the blue visible light is considered unsafe to the eyes. It can be divided into the short-wavelength blue region (SWB, 381–460 nm) and the long-wavelength blue region (LWB, 461–500 nm). Similarly to UV blocking ability, all films showed the ability to reduce blue light transmittance suggesting some protection against blue light, particularly when the amount of RSF was increased. 100CS/0RSF film showed SWB transmittance of 86%, while 70CS/30RSF was reduced to 80%. These results suggested that the CS/RSF blended films showed greater potential in the protection from UV-B, UV-A, and blue light than CS film.
### Table 1: SEM micrographs of surface and cross-section of CS/RSF films (×1000 magnification)

<table>
<thead>
<tr>
<th>Mass ratio of CS/RSF (w/w)</th>
<th>Surface</th>
<th>Cross-section</th>
</tr>
</thead>
<tbody>
<tr>
<td>100/0</td>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
</tr>
<tr>
<td>90/10</td>
<td><img src="image3.png" alt="Image" /></td>
<td><img src="image4.png" alt="Image" /></td>
</tr>
<tr>
<td>80/20</td>
<td><img src="image5.png" alt="Image" /></td>
<td><img src="image6.png" alt="Image" /></td>
</tr>
<tr>
<td>70/30</td>
<td><img src="image7.png" alt="Image" /></td>
<td><img src="image8.png" alt="Image" /></td>
</tr>
</tbody>
</table>

**Fig. 1:** FTIR spectra of RSF film, PEG400, CS film and CS/RSF films with indicated blend ratios
Table 2: Light transparency of CS/RSF films

<table>
<thead>
<tr>
<th>Mass ratio of CS/RSF (w/w)</th>
<th>Light transparency (%)±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>UV-B (280-315 nm)</td>
</tr>
<tr>
<td>100/0</td>
<td>27±2</td>
</tr>
<tr>
<td>90/10</td>
<td>17±0</td>
</tr>
<tr>
<td>80/20</td>
<td>10±1</td>
</tr>
<tr>
<td>70/30</td>
<td>12±2</td>
</tr>
</tbody>
</table>

SD: standard deviation, n = 3

Fig. 2: Light transparency of CS/RSF films with indicated blend ratios, error bars indicate SD for n = 3

Mechanical properties of CS/RSF films

The mechanical properties of contact lenses are important considerations with respect to durability and resistance to damage during handling. The stiffness and flexibility of contact lenses are expressed by Young's modulus and elongation at break, respectively. The Young's modulus of CS/RSF films was decreased by increasing RSF content, table 3. 100CS/0RSF film showed Young's modulus of 6 N/mm², while 70CS/30RSF was reduced to 3.8 N/mm². The maximum Young's modulus of the various commercial soft contact lenses materials are reported to be at 1.5 N/mm² [22, 23, 48, 64]. Clearly, the stiffness of CS/RSF films was slightly higher suggesting a more stiffness and thus are easier to handle and less likely to fold in on itself. Similarly, the RSF content affected the elongation at break. The elongations at break of 100CS/0RSF and 70CS/30RSF films were 135 and 113%, respectively (table 3). Nevertheless, all prepared blended films possessed the elongation at break of>50% which are considered to satisfy the flexibility requirement [48].

Water content of CS/RSF films

Water content is one of the key parameters to determine the comfort of wearing contact lenses wearing. Contact lenses with high water content would offer greater softness and comfortable wearing. According to FDA's classification, soft contact lenses with the water content of <50% by weight is considered as "low water content", while those with >50% by weight is considered as "high water content". All prepared CS/RSF films showed high water content (59 to 65% by weight) as shown in table 3. Nevertheless, the water content of CS/RSF films was slightly decreased as the content of silk fibroin increased. This phenomenon could be explained by the intermolecular hydrogen bonds between CS and RSF molecules, resulting in the reduced interaction between CS and water.

Table 3: Mechanical properties and water content of CS/RSF films

<table>
<thead>
<tr>
<th>Mass ratio of CS/RSF (w/w)</th>
<th>Young's modulus (N/mm²)±SD</th>
<th>Elongation at break (%)±SD</th>
<th>Water content (%)±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>100/0</td>
<td>6.0±1.2</td>
<td>135±17</td>
<td>65±1.34</td>
</tr>
<tr>
<td>90/10</td>
<td>5.4±0.6</td>
<td>116±28</td>
<td>62±1.38</td>
</tr>
<tr>
<td>80/20</td>
<td>4.6±1.1</td>
<td>111±26</td>
<td>59±1.35</td>
</tr>
<tr>
<td>70/30</td>
<td>3.8±0.5</td>
<td>113±33</td>
<td>59±0.92</td>
</tr>
</tbody>
</table>

SD: standard deviation, n = 3

Thermal properties of CS/RSF films

Thermal properties of CS/RSF films were investigated by DSC measurement as shown in fig. 3. Water evaporation temperature, glass transition temperature (Tg), thermal decomposition temperature were investigated. A broad endothermic peak below 110 °C observed in all CS/RSF films was attributed to moisture evaporation. With increasing RSF content, the height and area under endothermic peak were decreased indicating the reduction of moisture in the films. This observation correlated to the water content of the film, the water content of CS/RSF films decreased with increasing RSF content, table 3.
The Tg of 100CS/0RSF film was observed at 135 °C, while 70CS/30RSF film showed Tg at 122 °C. Interestingly, with increasing RSF content, the decreasing Tg was observed indicating the increased amorphous portion of CS/RSF film [65]. The increasing amorphous fraction could be attributed to a decrease in the film strength. This result was in agreement with their mechanical properties. The Young's modulus of CS/RSF films decreased with increasing RSF content. From DSC peaks, the thermal decomposition temperature of CS/RSF films was ~265-330 °C. The initial thermal decomposition temperature of CS/RSF films slightly increased from 265 to 270 °C with increasing RSF content indicating that RSF could slightly increase the thermal stability of CS/RSF films.

In addition, the thermal properties of the blended film were also confirmed by TGA. The initial weight loss of CS/RSF films at below 170 °C, ~5-20%, was due to water evaporation (fig. 4A). In accordance with DSC results, the moisture content of CS/RSF films decreased with increasing RSF contents. However, to determine the effect of RSF content on thermal decomposition temperature, the
TGA curves of CS/RSF films were adjusted to avoid the interference from the moisture (Fig. 4B). The thermal decomposition temperature of CS/RSF films was ~ 260-330 °C similar to DSC results. At the thermal decomposition temperature, the residual weight of CS/RSF films slightly increased with increasing RSF. This indicated that increasing RSF slightly increased the thermal stability of CS/RSF films. Furthermore, both DSC and TGA techniques revealed that all CS/RSF films possessed high thermal stability with the thermal decomposition temperature of >260 °C. This confirmed that CS/RSF films were able to be autoclaved at 121 °C without deterioration.

**Ion permeability of CS/RSF films**

Ion permeability of contact lenses is a critical variable for lens motion on the eye [66]. For sufficient on-eye-movement, typically, ion permeability of the lens should be greater than 12×10⁻⁶ cm²/min [67].

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**Oxygen permeability of CS/RSF films**

Oxygen permeability is an important parameter to characterize the contact lenses. Contact lenses with high oxygen permeability to the cornea tend to be safer, lower the risk of corneal hypoxia, and provide greater comfort of wearing. 100CS/0RSF and 70CS/30RSF contact lenses were successfully prepared by spinning casting method with 0.2 mm in thickness. The oxygen permeability of 100CS/0RSF contact lenses was 22 Barrers (10⁻¹¹ cm³/sec·cm Hg·mm). In contrast, the 70CS/30RSF contact lenses showed greater oxygen permeability of 26 Barrers. This result indicated that oxygen permeability of CS/RSF films slightly increased with higher amount of RSF because it could be strongly related to the polymorphism of the film [68]. As evident from DSC results, the amorphous portion of CS/RSF films slightly increased with increasing RSF content. Accordingly, oxygen permeability of CS/RSF contact lenses were shown to meet standards for use as daily disposable contact lenses as compared to the commercial contact lenses (10-33 Barrers) [20, 64].

**In vitro enzymatic degradation study**

Enzymatic degradation is a significant consideration in the design and quality control of the soft contact lenses materials. Biodegradable materials are not suitable for contact lenses application because the small residual may cause eye irritation. Thus, the stability of CS/RSF film in tear fluids containing important amounts of proteins and lysozyme is a crucial issue. The CS/RSF films are constructed from CS, which can be hydrolysed by the lysozyme presenting in tear fluids. Therefore, the remaining weight of CS/RSF was determined upon their incubation in the STF containing lysozyme. After incubation in the STF containing lysozyme for 14 d, only 70CS/30RSF film showed no degradation with percentage of remaining weight of 100% (table 4). In contrast, CS/RSF films at ratios of 100/0, 90/10, 80/20 (w/w) illustrated slight degradation as evidenced by from the remaining weight of 94, 97, and 99%, respectively. The degradation of CS/RSF films in the STF containing lysozyme increased by increasing the proportion of CS. This result could be attributed to a partial hydrolysis of CS by lysozyme [43, 51]. Nevertheless, all CS/RSF films incubated with STF containing lysozyme for 7 d showed no degradation, with remaining weight of 100%.

**Cytotoxicity study**

The cell viability of HCECs after incubation with CS/RSF films for 24 h was approximately 100% (see fig. 6) indicating that CS/RSF films are non-cytotoxic. In addition, non-cytotoxicity of CS/RSF films was further confirmed by microscopic observation (table 5). Upon treatment with CS/RSF films, the appearance of confuence HCECs showed no significant difference as compared to those treated without CS/RSF films (control). Similarly, after they were incubated with MTT, the appearance of HCECs treated with CS/RSF films showed similar morphology as those of control.

**Table 4: Percentage of the remaining weight of CS/RSF films after incubation in STF Containing lysozyme**

<table>
<thead>
<tr>
<th>Mass ratio of CS/RSF (w/w)</th>
<th>Remaining weight (%)±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 d</td>
</tr>
<tr>
<td>100/0</td>
<td>100±1</td>
</tr>
<tr>
<td>90/10</td>
<td>100±1</td>
</tr>
<tr>
<td>80/20</td>
<td>100±0</td>
</tr>
<tr>
<td>70/30</td>
<td>101±1</td>
</tr>
</tbody>
</table>

SD: standard deviation, n = 3
Table 5: Optical micrographs of HCECs after 24 h incubation with film and after 2 h incubation with MTT solution (×4 magnification)

<table>
<thead>
<tr>
<th>Mass ratio of CS/RSF (w/w)</th>
<th>After 24 h incubation with film</th>
<th>After 2 h incubation with MTT solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (without film)</td>
<td><img src="image1" alt="Control Image" /></td>
<td><img src="image2" alt="Control Image" /></td>
</tr>
<tr>
<td>100/0</td>
<td><img src="image3" alt="100/0 Image" /></td>
<td><img src="image4" alt="100/0 Image" /></td>
</tr>
<tr>
<td>90/10</td>
<td><img src="image5" alt="90/10 Image" /></td>
<td><img src="image6" alt="90/10 Image" /></td>
</tr>
<tr>
<td>80/20</td>
<td><img src="image7" alt="80/20 Image" /></td>
<td><img src="image8" alt="80/20 Image" /></td>
</tr>
<tr>
<td>70/30</td>
<td><img src="image9" alt="70/30 Image" /></td>
<td><img src="image10" alt="70/30 Image" /></td>
</tr>
</tbody>
</table>

Fig. 6: Cell viability of HCECs after exposed to CS/RSF films for 24 h, error bars indicate SD for n = 3
CONCLUSION
The CS/RSF films at ratios of 100/0, 90/10, 80/20 and 70/30 (w/w) showed high visible light transparency, smooth surface morphology and their cross-sections exhibited homogenous blending between CS and RSF without phase separation. With increasing RSF content, oxygen permeability, and thermal stability of the prepared films increased whereas the mechanical properties and water content of the prepared films slightly decreased. Moreover, all prepared films showed high thermal stability, high Young's modulus and elongation at break. In conclusion, all prepared films were softness with high strength characteristics, good oxygen and ion permeability, high water content, no cytotoxicity and no degradation in STF containing lysozyme for 7 d implying that prepared films were biocompatible and could promote the comfort for wearing without irritation and grittiness in the eyes. Therefore, CS/RSF films showed excellent physicochemical properties and non-cytotoxicity indicating their promising potential use as a biomaterial for daily disposable contact lenses-based ophthalmic drug delivery system.

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AUTHORS CONTRIBUTIONS
All the authors have contributed equally

CONFLICT OF INTERESTS
The author reports no conflicts of interest

REFERENCES