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A hyaluronic acid-binding contact lens with enhanced water retention

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ABSTRACT

Purpose: As a main component of an artificial tear or eyedrop, hyaluronic acid (HA) prolongs water retention, slows tear removal, improves tear film stability, reduces protein adsorption at the ocular surface and permits uninterrupted blinking. Here, we hypothesized that the contact lens modified with an HA-binding peptide (HABpep) could locally bind and concentrate exogenous HA present in eyedrops to the modified contact lens surface, which exhibited superior water retention.
Methods: To bind HA, a contact lens surface was covalently modified by HABpep with and without a poly(ethylene glycol) (PEG) spacer. Bound HA and its retention over time on the modified surfaces were evaluated by fluorescence measurements. A comparative water evaporation study was performed to determine water retention in an HA-bound contact lens.
Results: Fluorescence studies showed that the contact lens was successfully modified by HABpep with or without a PEG spacer, and HA bound to the contact lens surface. Furthermore, the bound HA via HABpep significantly reduced water loss from the modified contact lens.
Conclusion: HABpep strategies that locally bind and concentrate HA to create a thin coating of a therapeutic molecule on surfaces could provide physical and biological benefits to treat ocular surface dysfunction. The surface bound HA via HABpep enhanced water retention in the modified contact lens.

1. Introduction

Contact lenses offer an attractive and effective option for non-invasive sight correction. However, contact lenses, where the ocular surface and tear fluid work synergistically, often lack sufficient hydration and lubrication, hampering their integration with the eye and their function over time [1,2]. One of the common problems in people who wear contact lenses is discomfort due to insufficient wetting, lubrication and protein adsorption on the ocular surface [1,2]. Silicon hydrogel-based contact lenses were initially developed with the exception that higher oxygen permeability would allow extended wear without many of the problems evident at the time; however, when worn on a daily basis no significant improvement in wear comfort was achieved compared to comfort with traditional hydrogel contact lenses [3–6].

Techniques that are employed to keep hydrophobic silicon hydrogels hydrated and functional include plasma surface treatment and application of wetting agents [6,7]. However, none of these techniques can completely stop the movement of hydrophobic moieties to the contact lens-tear film interface causing the lens to become drier, less lubricated, and increasingly discomforting overtime, especially over the course of the day [1,2,6,8–10]. Polymer wetting or lubricating solutions including hyaluronic acid (HA) can effectively wet the ocular surface [7]. However, their low bioavailability or ocular residence time due to limited or no adherence to the contact lens or ocular surface necessitates frequent instillation (e.g. HA turnover time <10 min) [11,12], while more viscous artificial tears blur vision and interfere with blinking [13,14].

HA is a natural, anionic glycosaminoglycan of the extracellular matrix (ECM) found in many tissues; its therapeutic application extends to various medical specialties, including ophthalmology [15,16]. It is used as an important component of artificial tears to treat dry eye and in eye drops that accelerate healing after surgery or trauma [14–18]. Hygroscopic and viscoelastic properties of HA prolong water retention, slow tear removal, improve tear film stability at the ocular surface and permit uninterrupted blinking [19–21]. HA is also shown to reduce protein adsorption on the contact lens surface, improving the overall comfort of wearing contact lens [22]. Moreover, HA has many biological functions, including the reduction of inflammatory mediators [23] and the

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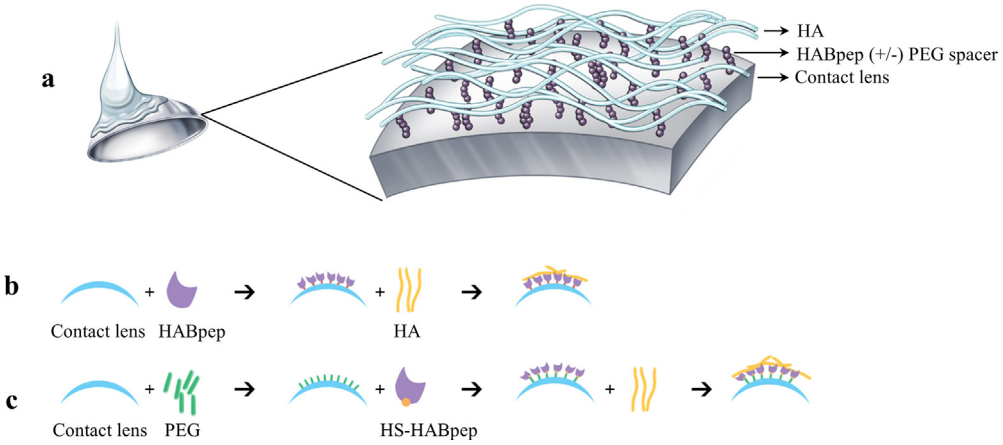

Fig. 1. Contact lens surface modification for HA binding via HABpep. (a) Schematic of contact lens surface modified with a HABpep designed to interact with and bind HA in surrounding fluid. (b) Contact lens modification with NHS functional groups, which, on reaction with primary amines of the HABpep, create HA binding sites. (c) Contact lens modification with amine functional groups, which on reaction with NHS of heterobifunctional PEG spacer, MAL-PEG-NHS, create thiol-reactive PEGylated HA binding sites. Subsequently, a thiolated HABpep is reacted to the maleimide functionality. On exposure to an HA solution, the HA binds to the peptide-polymer coating on the contact lens surface.

protection of cells from oxidative damage [24], which make HA an ideal therapeutic molecule to concentrate at the contact lens surfaces. Many ophthalmic products take advantage of HA's ability to enhance wettability and water retention (it can bind water 1000 times its mass via hydrogen bonding [25]), including multipurpose contact lens care solutions [26,27].

Our goal was to design a contact lens surface that retained a thin film of moisture and maintained a smooth, hydrated and lubricated ocular surface. Therefore, we developed a contact lens surface based on HA-binding peptide (HABpep), discovered through phage display [28], that was covalently bound to the surface (Fig. 1a–c). We hypothesized that modifying contact lenses with HABpep could localize HA to surfaces and would enhance water retention.

2. Methods

2.1. Preparation of HA-binding coatings on contact lens surfaces without a spacer

Contact lenses (PureVision®, balafilcon A, 36% water from Bausch and Lomb, NY) were cut using 4.5-mm biopsy punches. These pieces were added to 2-(*N*-morpholino)ethanesulfonic acid (MES) buffer solutions (pH 5.4) containing *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC, 3.0 mg/mL; Sigma–Aldrich, St. Louis) and *N*-hydroxysuccinimide or NHS (2.4 mg/mL; Sigma–Aldrich). After 10 min of activation, the samples were transferred to phosphate buffered saline (PBS, pH 7.4; Life Technologies, Grand Island, NY) solutions of either HABpep (GAHWQFNALTVR, ChinaPeptides, Shanghai, PRC) or fluorescein isothiocyanate-(FITC-)HABpep (ChinaPeptides) of varying concentrations (0, 0.005, 0.05, 0.5, 1.0 and 1.5 mg/mL) and stirred for 24 h at room temperature. The cut samples were vigorously washed with PBS to remove unreacted HABpep or FITC-HABpep.

2.2. Preparation of HA-binding coatings on contact lens surfaces with PEG spacer

HABpep was conjugated to the contact lenses pieces through a heterobifunctional PEG spacer. First, contact lens samples were modified with amine functional groups by stirring them in MES buffer solutions (pH 5.4) containing EDC (3.0 mg/mL) and NHS (2.4 mg/mL), followed by transferring them into a PBS buffer solution (pH 7.4) containing an excess of ethylene diamine (10.0 mg/mL). After 4 h of reaction time, contact lens samples were vigorously washed with PBS (pH 7.4). A heterobifunctional PEG spacer, Maleimide-PEG-*N*-hydroxysuccinimide (MAL-PEG-NHS, 3.5 kDa, JenKem Technology USA, Allen, TX), which has functionalities that are thiol- and amine-reactive, was dissolved to 5 mM in 50 mM sodium bicarbonate, pH 7.5, and added to the contact lens samples. The NHS groups were allowed to react with the amines on the contact lens surface for 30 min. Following thorough washes in PBS buffer to remove unreacted crosslinker, a 1.5 mM solution of cysteine-HABpep (CRRDDGAHWQFNALTVR) was added to the surface to react with maleimide groups for an additional 30 min. Surfaces were washed three times for 1 h with PBS to remove unreacted peptide, yielding contact lens surfaces with covalently attached HA-binding functionality.

2.3. Fluorescence visualization and measurements of the HA-bound contact lens surface

HABpep-modified contact lenses were added to a solution of HA-rhodamine or HA-fluorescein (Creative PEGWorks, Winston-Salem, NC) in sealed Eppendorf tubes and kept on a shaker for 24 h at ambient temperature. After washing three times with PBS for 24 h, fluorescence images were taken using a Zeiss Discovery V2 (Carl Zeiss AG, Jena, Germany) imaging microscope and processed with ImageJ (US National Institutes of Health [NIH], Bethesda, MD). To measure HA absorption or binding on both unmodified and modified contact lenses, the contact lens samples were submerged into 200 μL of fluorescently labeled HA in a 96-well round bottom plate and the fluorescence was measured by a plate reader. A standard curve was created using known HA concentrations. The following day, 150 μL of the HA soak solution from each well was relocated and the fluorescence was measured. HA concentration was calculated from 150 μL of the standard assay. To measure FITC-HABpep binding, the contact lenses were imaged on a Zeiss microscope. The HABpep treated lenses were rinsed vigorously and then imaged again. The brightness of a representative box was calculated with ImageJ and normalized to control, both before and after the treatment. To quantify HA binding versus HABpep, contact lenses were treated with 0, 0.005, 0.05, 0.5 and 1.0 mg/mL of HABpep and soaked in 1.0 mg/mL HA. To measure HA retention overtime, the contact lenses treated with various concentration ratios of HABpep and HA were soaked