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Matrix Metalloproteinase-Deactivating Contact Lens for Corneal Melting

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Abstract

Corneal melting is an uncontrolled, excessive degradation of cellular and extracellular components of the cornea. This potential cause of corneal blindness is caused by excessive expression of zinc-dependent matrix metalloproteinases (MMPs) and has no satisfying cure as of now. Herein, we introduce a novel therapeutic hydrogel which can be made into a contact lens to slow down the progression of corneal melting by deactivating MMPs. The hydrogel backbone is comprised of poly(2-hydroxyethyl methacrylate) (pHEMA), a main material for commercial contact lenses, and dipicolylamine (DPA) which has high affinity and selectivity towards zinc ion. Due to the high affinity towards zinc ions, the DPA-conjugated pHEMA (pDPA-HEMA) hydrogel selectively removes zinc ions from a physiological buffer and deactivates MMP-1, MMP-2 and MMP-9 within 2 hours. pDPA-HEMA hydrogel also effectively prevents degradation of porcine corneas by collagenase A, a zinc-dependent protease, whereas the corneas completely degrades within 15 hours when incubated with pHEMA hydrogel. The presence of pDPA-HEMA hydrogel does not affect the viability of keratocytes and corneal epithelial cells. Unlike the conventional MMP inhibitors (MMPI), the pDPA-HEMA hydrogel minimizes the risk of serious non-specific side effects, and provides a method to slow down the progression of corneal melting and other related ocular diseases.

Graphical Abstract

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ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website.

Detailed experimental methods, ¹H and ¹³C NMR spectra of DPA-HE and DPA-MA, LC/MS of DPA-MA, zinc assay, proliferation of HCECs and human keratocytes (PDF).

Keywords

zinc; dipicolylamine; hydrogel; contact lens; matrix metalloproteinases; corneal melting

Corneal melting, an uncontrolled excessive degradation of corneal tissue, is associated with various ocular diseases, such as ulcerative keratitis and autoimmune diseases (e.g., Stevens-Johnson Syndrome and rheumatoid arthritis).^{1–2} Ocular surgical procedures, such as cataract, glaucoma and LASIK surgeries, affecting millions of patients each year, significantly increase the risk of corneal melting.³ In addition, chemical burns to the cornea, affecting 30,000 workers annually in the United States, often progress to corneal melting.^{4–5} When treated improperly, corneal melting results in cornea perforation and can lead to eventual vision loss.⁶ Current treatments include topical application of steroidal anti-inflammatory drugs, application of tissue adhesives or amniotic membrane transplantation, and corneal transplantation.⁷ To date, however, there is no satisfying cure. Recently, it was found that the tear film of corneal melting patients consisted of an abnormally high accumulation of matrix metalloproteinases (MMPs).^{1, 7} MMPs are zinc-dependent enzymes with zinc-binding sites within the enzymatic domain.⁸ MMPs are essential proteins for the renewal of extracellular matrices (ECMs), and abnormal production of MMP has been connected to various adverse health conditions including rheumatoid arthritis,⁹ cardiovascular diseases¹⁰ and cancer metastasis.^{10–11} Although the detailed profiles of elevated MMPs depend on the etiology of corneal melting, application of MMP inhibitors (MMPi) is considered a potential treatment.^{12–13} Most MMPi deactivate MMPs by binding to the zinc in the active site.¹⁴ However, preventing the systemic circulation of MMPi is nearly impossible and results in non-specific actions of MMPs, which can be the source of serious side effects such as musculoskeletal syndrome.^{15–16}

Here, we introduce a poly(2-hydroxyethyl methacrylate) (pHEMA) hydrogel that can be made into a contact lens with a zinc-absorbing capacity to treat corneal melting (Figure 1). Sequestration of zinc ions by the hydrogel will lead to the loss of zinc ions from MMPs and consequent deactivation of MMPs. Zinc absorption by the pHEMA hydrogel is achieved by the covalent conjugation of dipicolylamine (DPA) which has a selective binding affinity towards a zinc ion (up to $K_d = 10^{-11}$ M)¹⁷ and does not associate strongly with most physiological metal ions such as Ca^{2+} , Mg^{2+} , Na^+ and K^+ .¹⁷ In previous studies, the selectivity of DPA towards zinc has been used as a non-toxic zinc indicator: DPA-based fluorescent probes (e.g., Newport Green) selectively label labile zinc in viable beta-cells,¹⁸ measure Zn binding to amyloid-beta (Ab)¹⁹ and quantitatively assess zinc concentration

under various physiological conditions.^{20–21} In addition, zinc-DPA complexes have been used in biosensing and separation of bacteria.²²

This novel treatment method is fundamentally different from previous treatments for MMP-associated diseases in that MMPs are deactivated by the absorption of zinc ions by the contact lens instead of the release of therapeutic molecules (e.g., MMPi). This aspect of the treatment substantially reduces the risk of detrimental side effects associated with the circulation and non-specific actions of therapeutics.

To prepare the DPA-conjugated pHEMA (pDPA-HEMA) hydrogel, we synthesized a monomer, DPA-methacrylate (DPA-MA) by reacting DPA-modified precursor (DPA-HE, Figures S1 and S2, Supporting Information) with methacryloyl chloride (Figures S3–S5, Supporting Information). pDPA-HEMA hydrogel was formed by mixing DPA-MA, HEMA, ethylene glycol dimethacrylate (EGDMA, a crosslinker) and Irgacure 2959 (a photoinitiator) in the presence of water, followed by curing under UV ($\lambda_{\text{max}} = 365 \text{ nm}$, 400 W) for 1 hour (Figure 2a).²³ Incorporation of DPA in pHEMA resulted in an optically transparent hydrogel with a slightly yellow tint (Figure 2b). Although the transmittance of pDPA-HEMA hydrogel was lower than pHEMA hydrogel throughout the entire visible spectrum, it was higher than 0.8 above 436 nm, making it suitable as a contact lens (Figure 2c).

We first demonstrated the specific zinc-absorbing capacity of pDPA-HEMA hydrogel (Figure 3a). pDPA-HEMA hydrogels with varying quantities of DPA were incubated in an aqueous zinc solution, and the amount of zinc ions remaining in the solution was measured over different DPA concentrations in the hydrogel. As DPA content increased, more zinc ions were absorbed by the hydrogel. Since the number of DPA on the hydrogel surface was only in the order of pmoles (see the Supporting Information for the detailed order of magnitude analysis), and 1 μmole of zinc ions were removed by the hydrogel, we can assume that the binding of zinc ions to DPA occurred not only on the surface but also within the hydrogel. Due to the high selectivity of DPA towards zinc, the zinc-absorbing capacity of pDPA-HEMA hydrogel was unaffected by the presence of calcium at a 10 times higher concentration. pHEMA hydrogel (without DPA) removed a small fraction (30%) of zinc ions, likely due to the weak non-specific interaction between zinc ions and the hydroxyl groups of pHEMA. However, the amount of removed zinc ions by pHEMA hydrogel was significantly lower than that by pDPA-HEMA hydrogel.

Figure 3b and 3c show scanning electron microscope (SEM) images of small fragments of lyophilized hydrogels after zinc absorption with the elemental analysis performed by energy-dispersive X-ray spectroscopy (EDS). The profile of zinc signal (green line) along the red line of the SEM image perfectly matched the profile of the pDPA-HEMA hydrogel (Figure 3b), indicating that the disappearance of zinc ions from the solution was caused by the accumulation of zinc ions in the pDPA-HEMA hydrogel. No significant amount of zinc was detected on the pHEMA hydrogel (Figure 3c). When the entire field of the SEM image of the pDPA-HEMA hydrogel was scanned for zinc in the EDS mode, the distribution of zinc was shown to overlap with the zinc in the SEM image (Figure 3d). A negligible amount of zinc was detected in the pHEMA hydrogel (Figure 3e). Combined with the zinc assay,

these results prove that the inclusion of DPA renders the pHEMA hydrogel with zinc-absorbing capacity.

Subsequently, we tested whether removing zinc from the media using pDPA-HEMA hydrogels would lead to the reduction of MMP activities. Interaction between zinc and zinc-binding sites in MMPs is based on the coordination bonds²⁴ and is reversible with the off-rate of around 3 hours²⁵; if the surrounding has a low zinc concentration, MMPs eventually lose the zinc ions from the zinc-binding sites and are deactivated. MMP-1, MMP-2 and MMP-9 were chosen as test subjects because they are the major enzymes that are responsible for corneal melting.² MMPs were incubated with the hydrogels during the 2 hours of MMP activation by 4-aminophenylmercuric acetate (APMA). Figure 4a and Figure S6 clearly demonstrate that there is a significant reduction in enzymatic activities of all MMPs that were tested, whereas pHEMA hydrogel (without DPA) had no effects. pDPA-HEMA hydrogel deactivated MMP-2 more effectively than MMP-1 or MMP-9. Since the basic mechanism of MMP deactivation is the loss of zinc ions from the zinc-binding sites of the enzyme, we expect this hydrogel can be applied to all other zinc-dependent MMPs, although the effectiveness of deactivation may differ for different MMPs.

An *ex vivo* cornea degradation model was used to simulate the corneal melting²⁶: 1) Porcine corneas have been widely used as a model system for various potential treatments for human corneas^{27–28}; and 2) Collagenase A is a zinc-dependent protease, a bacterial analogue of human MMPs and has been widely used as a model MMP due to its ready availability^{26, 29}. When freshly obtained porcine cornea was incubated in 0.3 % (w/v) collagenase A solution with pHEMA and pDPA-HEMA hydrogels, the corneas completely degraded within 12 and 15 hours, respectively (Figure 4b). When the collagenase A concentration was reduced to 0.15 % (w/v), the degradation time slightly increased to 15 hours for pHEMA hydrogel while the corneas did not degrade completely even after 72 hours for pDPA-HEMA hydrogel. Corneal melting is a slowly progressing symptom, and the concentration of MMPs in the tear film of the corneal melting patients is in the order of a few nanograms per milliliter,² which is orders of magnitude lower than the collagenase A concentrations in the current study. Therefore, it is expected that pDPA-HEMA hydrogels will be highly effective in preventing corneal melting *in vivo*.

To observe the microscopic changes taking place within the degraded cornea tissue, the tissues were fixed before the complete degradation (i.e., 8 hours incubation in 0.15 % collagenase A solutions). The untreated cornea tissue shows a clear stratified epithelium and a stroma with a well-packed ECM (Figure 4c). When the cornea was incubated in collagenase A without any hydrogel, the cornea tissue lost the entire epithelium and had many small holes in the exterior of the residual tissue (Figure 4d). In addition, the H&E staining was much weaker than the untreated cornea tissue, likely due to much degradation of the ECM. The cornea tissue incubated in collagenase A with pHEMA hydrogel had similar structures (Figure 4e). However, the tissues incubated in collagenase A with pDPA-HEMA hydrogel remained mostly intact except for some damage on the epithelium (Figure 4f). The results from the cornea degradation study and histological analyses confirm that pDPA-HEMA hydrogel prevents the degradation of corneal ECM by deactivating the collagenase A.

It is important that the removal of zinc ions from the cornea and tear film does not cause cytotoxicity. Cytotoxicity was tested on human corneal epithelial cells (HCECs) and human keratocytes because these cells are most likely to be impacted by the alterations in zinc concentration by the hydrogels. The cells were cultured in 24-well tissue culture plates and were exposed to the hydrogels through semi-permeable membranes. For both HCECs and human keratocytes, no significant cytotoxicity by pDPA-HEMA hydrogels was observed for 48 hours (Figure S7, Supporting Information). We chose relatively early time points (24 and 48 hours) for the cytotoxicity tests because the contact lenses in general are not worn for an extended period. The pDPA-HEMA hydrogel did not cause any significant level of apoptosis either (Figure S8, Supporting Information)

The potential treatment using the pDPA-HEMA contact lens for corneal melting is more advantageous compared to the conventional treatments for the following reasons: 1) Unlike the currently available MMPi, there is no systemic circulation of the zinc-targeting molecules because the DPA molecules are covalently conjugated in the polymer network of the contact lens. Therefore, the therapeutic effects and the associated side effects will be limited to the affected cornea; and 2) DPA selectively binds to a zinc ion among all (biological) metal ions, which further decreases the chance of side effects resulting from the depletion of other metal ions.

pDPA-HEMA hydrogels can be synthesized on a large scale at a low cost, and can be treated by the same standard sterilization techniques as pHEMA hydrogels, which ensures the practicality of this material.

In conclusion, pDPA-HEMA hydrogel was able to deactivate MMPs by selectively removing zinc ions. Our results suggest that the pDPA-HEMA hydrogel can be a useful therapeutic option for treating corneal melting.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.
Schema of deactivation of MMPs by the pDPA-HEMA-based contact lens

Figure 2.

(a) Photopolymerization of pDPA-HEMA: DPAs are conjugated to the polymeric network of HEMA crosslinked by EGDMA. (b) Photographs of pDPA-HEMA (5 % of DPA with HEMA) before (top) and after (bottom) UV irradiation for 1 hour; the resulting hydrogel was transparent with a slight yellow tint. (c) Transmittance spectrum of pHEMA and pDPA-HEMA hydrogels.

Figure 3.

(a) Zinc assay showing the specific zinc-absorbing capacity of pDPA-HEMA hydrogel in the absence (blue line) and presence (red line) of calcium: hydrogels (8 mm in diameter, 2 mm in thickness) were immersed in 1.0 mL of 1.0 mM ZnCl₂ or 1.0 mL of 1.0 mM ZnCl₂ and 10 mM CaCl₂ for 1 hour, and the amount of zinc ions remaining in the solution was measured using a fluorescent indicator for zinc. 3.75 and 7.5 μmoles of DPA in the pHEMA hydrogel correspond to 2.5 and 5% DPA. (b,c) SEM images of small pieces of (b) pDPA-HEMA and (c) pHEMA hydrogels after the zinc absorption study (the green lines in b and c

represent the amount of zinc obtained by EDS along the red lines). (d,e) Zinc elemental mapping of (b) and (c) after the zinc absorption study; green pixels represent Zn. (scale bar = 100 μm)

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Figure 4.

(a) Deactivation of MMPs by pHEMA and pDPA-HEMA hydrogels. x-axis indicates the amount of DPA in the pHEMA hydrogel. 7.5 and 15 μ moles of DPA correspond to 5 and 10% DPA in the hydrogel, respectively. (b) *Ex vivo* degradation of porcine cornea: the time it takes for the complete degradation of porcine cornea was measured. * and ** denote $p < 0.05$ and $p < 0.01$, respectively ($n = 4$). DPA concentration was 10% for pDPA-HEMA. (c-f) Optical micrographs of H&E stained histology sections: (c) untreated cornea, corneas

incubated in 0.15 % collagenase A solution for 8 hours (d) without any hydrogel, (e) with pHEMA hydrogel and (f) with pDPA-HEMA hydrogel. Scale bar = 200 μm .

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