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Adjustable release of mitomycin C for inhibition of scar tissue formation after filtration surgery

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Abstract

The aim of this study is to demonstrate a drug delivery system with the capacity to adjust the release of mitomycin C (MMC), based on polymer composition, and inhibit fibroblast proliferation to a better effect than is currently used in glaucoma filtration surgery. The polymer used in this work is made from the oligosaccharide cyclodextrin, from which others and we have demonstrated adjustable release of small molecule drugs due to specific molecular interactions or “affinity” between drug and the cyclodextrin polymer. To adjust release rate, cyclodextrin polymers were synthesized in either dimethylformamide (DMF) or dimethyl sulfoxide, (DMSO) at a crosslinking ratio of 1:0.16 or 1:0.32 (molecule of glucose: molecule of crosslinker). The polymers were then loaded with mitomycin C, dried, and release evaluated in a physiological environment. Drug release was determined by visible spectroscopy. Released aliquots of mitomycin C were incubated with 3T3 fibroblast cells to determine cytotoxic or inhibitory effect through a cell proliferation assay. We show that by using affinity between drug and polymer, we can adjust MMC release rates to be slower and more sustained than from conventional, diffusion-only polymers, for both the DMF polymers ($p = 0.00526$) and the DMSO polymers ($p = 0.0113$). The incorporated and released MMC maintains inhibition of fibroblast proliferation much longer than is possible with a one-time application. Affinity polymers with 1:0.16 and 1:0.32 crosslink ratio showed significant inhibition of proliferation for up to 100 h ($p = 0.018$ and $p = 0.014$ respectively). The use of our controlled drug delivery technology applied after surgery could have a greater therapeutic impact than the current one-time applications of MMC.

Keywords

cyclodextrin; mitomycin; controlled release; sustained release; anti-proliferative; drug delivery; glaucoma; filtration surgery

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None.

1. Introduction

A common side effect of filtration surgery is excessive scar tissue formation, especially near the introduced bleb. While some scar tissue formation is expected, too much scar tissue hinders other aqueous humor drainage channels, making the surgery ineffective (Coleman, 2012; Olali et al., 2011). Historically, mitomycin C (MMC) is used to reduce fibroblast proliferation, and minimize undesired blocking of the trabecular meshwork, in glaucoma patients (Lee et al., 2009). MMC is a bifunctional alkylating agent that works as an anti-proliferative drug by binding to guanine residues in DNA and inhibiting DNA synthesis (Beckers et al., 2003; Hung et al., 1995; Lee et al., 2009). Ophthalmologic treatments using MMC aim to reduce excess cell proliferation. In typical applications, concentrations of MMC near 0.5 mg/mL were soaked into a poly-vinyl alcohol (PVA) sponge and left to sit on the sclera for a short duration (al-Hazmi et al., 1998; Cordeiro et al., 1997). Although this only extended delivery from instantaneous to over a period of minutes, the results were promising, demonstrating the appeal of longer-term delivery. However, administration through sponges caused very fast, non-linear, release of drug onto the eye. The mechanism of drug release in this case is solely through drug diffusion, which is common for most drug delivery systems but found to be inappropriate for this application. Reports showed that the short-term, high MMC doses available through this “diffusion-only” administration produced side effects such as defects in the corneal epithelium, filtering bleb leakage, low intraocular pressure (IOP), and inflammation (Duan et al., 2012). An alternative delivery treatment of MMC uses a subconjunctival injection of the drug administered 1–5 days before filtration surgery (Hung et al., 1995). The injection is a single dose of 1–3 μ g total, an amount chosen to avoid high concentrations of MMC, which lead to systemic toxicity. The results are promising for reducing ciliary body toxicity, long-term hypotony of less than 5 mmHg, and macular changes for patients that have a relatively normal conjunctiva. However, those with a thin conjunctiva or thick conjunctival scarring were found to not benefit much from a one-time administration of MMC (Rubinfeld et al., 1992).

Titration studies, including a copious eye-washing step after the 5 $\times$ 7 mm drug-loaded sponge was removed, showed no correlation between MMC delivery parameters and IOP outcome (Lee et al., 2009). The studies tested drug-loading concentrations between 0.2 and 0.5 mg/mL, and exposure times between 0.5 and 5 min. However there was no statistically significant difference in outcomes, the only qualitative observation was that exposure time most likely has more of an effect than drug loading concentration (Lee et al., 2009). A probable explanation is that the high initial release dose of MMC, regardless of loading or exposure, had the highest impact on the treatment. Since the diffusion-based delivery is rapid and non-linear, subsequent delivered drug would be at a substantially lower concentration. It would therefore be difficult to notice any difference in tissue response from changes in drug loading concentration. A delivery system, which could decrease the initial dose and increase subsequent delivery doses, might therefore show a better long-term outcome than was seen with these sponges.

Here we propose a new system for MMC delivery, using a polymer, which has an additional feature beyond diffusion-based systems, namely the ability to prolong the release of drug using chemically designed affinities between polymer and drug. Briefly, affinity is

characterized by the ability of a molecule to bind to a substrate through molecular interactions. Non-covalent interactions are largely what determine the strength of these affinities, in particular hydrophobic interactions, which are used in this system (Li and Loh, 2008). The increase in binding strength proportionately slows the release of bound therapeutics, and results in a more sustained release. Diffusion-only systems, however, release agents at a rate almost entirely based on concentration gradients, so it is difficult to adjust the release rate of such systems beyond an order of magnitude (Wang and von Recum, 2011).

Our approach for the prolonged release of MMC is to complex the drug with an insoluble, crosslinked cyclodextrin polymer. Cyclodextrin (CD) is an organic, cyclic oligosaccharide molecule. Specifically we used gamma-CD herein, in which eight glucose molecules are covalently bound to form a toroid structure. This toroid structure has a hydrophilic exterior and a relatively hydrophobic interior. Even slightly hydrophobic drugs can form inclusion complexes with the CD interior cavity via affinity interactions (Thatiparti et al., 2011, 2010). We have previously shown that with CD polymers, which have a specific affinity to hydrophobic drugs, we can achieve slow, sustained release rates far beyond that of diffusion alone (Thatiparti et al., 2011, 2010; Wang and von Recum, 2011). In the present work we tested MMC release from such an affinity-based polymer to prolong the release of the drug while maintaining inhibition of fibroblast proliferation.

Although there have been studies to demonstrate that MMC has an affinity to cyclodextrins (Bekers et al., 1988, 1991) this study is the first that focuses on controlled delivery of MMC using these affinity interactions. Due to the capacity of these affinity-based materials to deliver therapeutics in a biologically relevant time frame (cell proliferation is on the order of days), we believe these polymers serve as a better alternative to bolus injections or to the sponge delivery method (Cordeiro et al., 1997; Hung et al., 1995). A product showing dose-controlled delivery of MMC at therapeutic rates could be applied clinically in a number of ways, such as a scleral buckle, an injectable depot, or a cyclodextrin-polymer contact lens.

2. Materials and methods

Approximately 1 g of gamma-cyclodextrin (CD) (Batch CYL-3347, Cyclolab) was dissolved in 4 mL of N,N dimethylformamide (DMF, Acroseal) or dimethyl sulfoxide (DMSO, Fisher Scientific) in glass sample vials of 20 mL at room temperature. Dextran (Polysciences) disks were made in DMSO using identical volumes. Varying quantities of hexamethylene diisocyanate (HDI, Sigma-Aldrich) were added to the CD containing vials (at ratios of either 1:0.32 or 1:0.16). Samples were vortexed and homogenized in order to ensure proper mixing. Upon complete dissolution, the CD reactions were poured into Teflon Petri dishes (PTFE Low 25 mL, Fisher Scientific) and heated at 70 °C for 2 h. After heating, the CD was allowed to cool to room temperature, and circular disks were cut out using an 8 mm diameter stainless steel core punch (Osborne Arch Punch). The circular, 8 mm diameter, disk shape will serve as an easy comparison to the 5 × 7 mm PVA sponge method described in the introduction. Seeing that the “bleb” is in a circular shape, we thought it would be ideal for the drug delivery vehicle to be circular in order to focus the delivery to the targeted area. To remove unreacted HDI and CD from the crosslinked polymers, DMF prepared samples

were incubated in DMF for 24 h, and DMSO-prepared samples were incubated in DMSO for 24 h. The polymer disks were then incubated in 50:50 organic solvent, respective to the solvent used during synthesis, and deionized water for 24 h. For the last wash, polymer disks were incubated in 100% deionized water for 24 h. After washing, polymer disks were dried at room temperature for 48–72 h, or until there was no change in dry weight measured.

Polymer disks were swelled in water, and in their respective synthesis solvents (DMF, or DMSO) for 24 h and weighed. The disks were then washed in a manner identical to the protocol described in the previous section, dried for 72 h and then weighed. The dry disk mass after swelling was compared to the dry disk mass before swelling. In order to ensure that excess solvent was removed, all disks needed to show less than a 1% change in dry mass weight before and after swelling to be included in the study. Swelling was calculated using the following equation $(W_1 - W_2)/W_2$. The swelling results will allow for theoretically calculated amounts of drug that can be hydrodynamically loaded into disks using different solvents.

The loading solution for all studies was 0.4 mg/mL mitomycin C (MMC, Santa Cruz) in 1 mL of deionized water, which is the maximum concentration of MMC soluble in water (Georgopoulos et al., 2002). Dried polymer disks were incubated with the MMC loading solution at 4 °C, the temperature at which MMC is most stable in water, on a tabletop rotating mixer for 4 days. Polymer disks were then dried for 72 h at room temperature, weighed, and then used for the drug release study. In the case of drug loading, it is important that the solvent is removed, via washing and drying, in order to give the drug a driving force to form an inclusion complex with the CD. If excess solvent were left in the gel, thermodynamically the drug would have less of a driving force to leave the solvent and complex with CD.

MMC loaded polymer disks were placed in phosphate buffered saline (PBS) with 0.5% Tween 20 in an “infinite sink” release system, meaning all of the release volume is removed and replaced with fresh solution at each time point. Tween 20 is a common surfactant used in *in vitro* drug release studies to mimic aspects of *in vivo* biological conditions (Thatiparti et al., 2011, 2010). Specifically, while *in vivo* conditions are very complex, Tween 20 can be used to add a hydrophobic element to the buffer solution, representing the hydrophobic sink that complex biological entities such as chylomicrons, lipoproteins and even albumin provides. This simple addition thereby makes a solution more physiologically relevant in regards to drug partitioning and drug release kinetics. The release was performed at 37 °C in an incubator shaker at 60 RPM, for a total of 7 days. Release into an agitated solution is more physiologically relevant since the body undergoes high fluid turnover as well as micro and macro motion. Agitation at 60 RPM is within the range of typical conditions used, as it also represents the frequency of the human heart rate. Release samples were taken at 0, 0.5, 2.5, 4.5, 6.5, 8.5, 10.5, 12.5, 24.5, 36.5, 48.5, 60.5, 72.5, 84.5, 96.5, 108.5, 120.5, 132.5, 144.5, 156.5, and 168.5 h, with a total release volume of 1 mL for each aliquot, and stored at 4 °C immediately. The total time frame for taking samples, as well as the frequency, was chosen based on how quickly the drug released from the CD and dextran disks. The goal in any such study is to take samples frequently enough to ensure high sink conditions, but not so frequently that handling of the samples might artificially change detected released. As

such, samples were taken more frequently in the earliest time points and less frequently at later time points. All released aliquot concentrations were analyzed together by a spectrophotometer at 364 nm, and compared to standards of known drug concentration. All studies were done in triplicate, with quantitative data shown as an average with a range of standard deviations.

Cell proliferation studies were performed on NIH 3T3 fibroblast cells (ATCC). A 48-well plate was seeded with approximately 5000 3T3 cells 12 h in advance.

An individual well of cells was then incubated with each release MMC aliquot, and the cells were then incubated for 72 h further. All samples were done in triplicate, and compared to cells grown in 20% (v/v) medium supplemented with 80% (v/v) of an “empty” delivery aliquot, consisting of the PBS buffer and 0.5% Tween 20 release buffer. Due to drug release in a solution other than cell culture medium, all MMC release aliquots were diluted to 80%, by volume, of the original concentration in normal growth media to ensure proper growth of cells. Normal growth media is Dulbecco’s Modification of Eagle’s Medium (HyClone) supplemented with 10%, by volume, fetal bovine serum (Gibco), and 1%, by volume, penicillin-streptomycin (HyClone). Addition of serum in growth conditions is required for cell proliferation and maintenance of cell osmotic pressure. The creation of a control well containing a combination of growth medium and an “empty” delivery aliquot ensure that background cell proliferation occurs in all conditions. On the third day, cell viability was determined using an MTS Assay (Promega, CellTiter Aqueous One, Protocol Part# TB245). The CellTiter Assay is a colorimetric method that uses a tetrazolium reagent, which is bio-reduced by living cells. Once reduced, the reagent becomes a formazan product that is soluble in aqueous solutions and has color with an absorbance at 490 nm. The change in absorbance values at 490 nm has a direct correlation with mitochondria activity, and can be used to assess the number of living cells present. For our studies, the MTS assay solution was added to each well of cells that were previously incubated with released MMC aliquots. Any color change was quantified by spectrophotometer (BioTek, Synergy H1 Microplate Reader) at 490 nm and compared to the “empty” aliquot controls. Separately, a minimum inhibitory concentration of MMC was determined by adding serial concentrations of MMC to 3T3 cells to generate an inhibition/proliferation profile.

For Scanning Electron Microscopy images, dried polymer samples were placed on carbon conductive double-sided tape and sputter-coated with palladium approximately 1 nm thick. The surface morphology of the polymers was analyzed with a Hitachi S450 Scanning Electron Microscope using a 5 kV accelerating voltage.

Fourier Transform Infrared Spectroscopy (FTIR) analysis was performed on air-dried polymer samples using a Bio-Rad FTS 575C spectrophotometer (Hercules, CA). This analysis serves as additional method to verify that the drug was incorporated into both CD and dextran disks. Polymers were ground into a potassium bromide pellet prior to analysis, and spectra were taken over the range of 600–4000 cm^{-1} . FTIR detects resonant frequencies of samples to determine the presence of chemical bonds. In order to detect such frequencies, the sample must be very thin, and as transparent as possible in the infrared spectrum, in order to give a clear spectrum (Merritt et al., 2012). Potassium bromide is spectrally

transparent in much of the infrared region, and allows one to create a mostly transparent pellet with small amounts of sample to decrease noise in the reading. Incorporation of amine groups was used to verify presence of MMC in the loaded samples.

All studies were done in triplicate, with quantitative data shown as an average with a range of standard deviations. Additionally, we ran Student's *t*-test to compare MMC release amounts, and differences in cell proliferation when exposed to release aliquots from either dextran or CD polymers loaded with MMC.

3. Results

Polymer synthesis parameters are compared below (Table 1), where the crosslink ratio is a molar ratio between one glucose unit in a CD (or dextran) molecule to one mole of HDI crosslinker. Dextran was used as a diffusion-only release control because it is a linear polysaccharide, with therefore a similar chemical makeup to cyclodextrin, but without the cyclic structure, and thus without the capacity for drug inclusion formation with the CD core. However, dextran is not soluble in DMF, so all dextran polymers were made in DMSO. The modifications in solvent and crosslinking ratio gave rise to polymers with different swelling and MMC release rates, which is shown in Fig. 4. The schematic in Fig. 1 shows MMC inclusion complex formation with a drug depot made from an insoluble polymer of CD.

Fig. 2 shows a low magnification view of dried CD disks.

Fig. 3 shows scanning electron microscope images of the different types of polymers tested. The 3,000 $\times$ magnification showed results that were representative of the polymer surface morphology (scale bar = 10 μ m). These images can be used to get qualitative information on polymer texture and any types of irregularities on the surface.

The CD and dextran polymers were synthesized in two different organic solvents, and show a higher degree of swelling in both of the organic solvents than in water (Fig. 4). Since MMC is hydrodynamically loaded into polymer disks, it is important to know swelling behavior differences of the different materials. Changes in polymer swelling will give information that can allow future control of the amount of drug loaded. Unfortunately, MMC is not stable in either DMF or DMSO; so all loading in this work was done in water. The CD (DMSO) polymer had the look of a dehydrated sponge under electron microscopy; lending credence to the belief that the solvent used led to macroscopic porosity in addition to the affinity-based properties. The CD (DMF) polymer had a less porous look, giving the impression of a more mechanically robust material. This is in line with the results of the swelling study tests, since the CD (DMSO) polymers swelled more than the CD (DMF) polymers tested. The dextran polymer also appeared to have pores on the surface, and presumably present throughout the material, which could serve as a factor for these materials having higher swelling ratios than either of the CD polymers.

Periodic release aliquots were taken from MMC loaded samples evaluated under “infinite sink” conditions described in the methods section. Both the amount of drug in each release aliquot, and the cumulative amount of drug released (sum of all previous release aliquots)

was plotted. The cumulative release data is shown in Fig. 5A, graphed as a percentage of total drug released, where the maximum drug released at 172 h is considered the maximum amount of drug released from the disk. This method of determining the maximum drug released was chosen because there was no detectable change in concentration of MMC released during the last 72 h of the experiment for all samples (and the samples had minimal residual blue color, indicating little to no residual loaded drug). Also, the composition of the release buffer is similar enough to the loading buffer, in which MMC is highly soluble, and allows us to assume that no drug is trapped in the disk due to partitioning. Total amounts of drug loaded are shown in Table 2.

From Fig. 5 we observe a rapid percentage release of MMC from the dextran polymers, and a slower, more sustained percentage of released MMC from CD polymers for the first 50–75 h. These differences are consistent regardless of crosslink ratio or solvent used. Qualitatively, polymers formed in DMF had a slower release than ones formed in DMSO, and polymers with 1:0.32 crosslink ratio showed slower release than ones with a 1:0.16 crosslink ratio. The CD polymers made in DMF, with a crosslink ratio of 1:0.32, show the most prolonged cumulative release out of all the samples tested.

The cumulative release data from Fig. 5 was re-plotted to show mass released in each release aliquot (Fig. 6). Standard deviations are shown in Fig. 5. They are not added to Fig. 6, due to the log scale of the *y*-axis. This plot confirms some initial burst release from all polymers at early time points. Although all materials were prepared under identical loading conditions the dextran polymers showed a lower total loading amount than the CD polymers, most likely due to an inability to form affinity inclusions. Both due to this inability to form inclusions, and the lower total loading, the dextran disks were quickly emptied and showed less long-term release than the affinity-based CD disks. Also the CD polymers made in DMF were confirmed to show more overall release at each time point than CD polymers made with DMSO. However, the release profiles between CD polymers made in the two different solvents were found statistically insignificant using a confidence interval of 95%. All CD drug release samples were found to have significantly more extended release of MMC than the dextran samples for the first 100 h of release. The resulting *p*-values were 0.00526 and 0.0113 for DMF-made and DMSO-made CD polymers respectively when compared to dextran polymers.

As described before, inhibition of NIH 3T3 cell proliferation was tested using an MTS assay. The purpose of the assay is to show the number of live cells present after incubation with aliquots of released MMC. The cell proliferation assays in Fig. 7 show the proliferation results of fibroblast cells incubated with released MMC at each time point of the release study. Cells were incubated for 72 h with individual release aliquots of MMC from the different CD or dextran polymer formulation. The results show that released dosages consistently inhibit fibroblast proliferation over the first 50–60 h of release from the CD polymer disks made with 1:0.16 crosslink ratio, and over the first 100 h from CD disks made with a 1:0.32 crosslink ratio. However, in the first 12 h, the released MMC from dextran polymers already start to lose efficacy.

The cell proliferation results showed no statistical difference in inhibition of proliferation from the DMF-made polymers than from DMSO-made polymers. However, the DMF-made polymers inhibited proliferation significantly more than the dextran polymers ($p = 0.018$), as did the DMSO-made polymers when compared to dextran polymers ($p = 0.014$).

To demonstrate the sensitivity of this cell proliferation model to MMC we attempted to determine an effective concentration range, which can be used to define dose-controlled release target values. Fig. 8 shows a change in cell proliferation values when incubated with different MMC concentrations for 72 h. For all concentrations tested, there is an initial amount of 1000 3T3 cells seeded. In that time period, the cells grown in the presence of release buffer containing no MMC is approximately 8000, which is consistent with 1000 cells doubling approximately every 24 h. Based on this figure the effective concentration needed to stop approximately half of the maximum proliferation, or the IC₅₀, is approximately 0.4 $\mu\text{g/mL}$, which is consistent with the amount of MMC released from CD disks for the first 100 h of the study.

Visually, the MMC loaded dextran and cyclodextrin polymers appeared blue, due to the blue/violet color of the MMC. However, spectral analysis was used to confirm MMC inclusion into the polymer (presence of amines) and was also used to demonstrate formation of the urethane bonds through isocyanate coupling (data not shown).

4. Discussion

Both dextran polymer formulations released a majority of drug within 2.5 h. The release was very fast, and plateaued near 100% cumulative release at approximately 12 h. This was expected because the drug was expected to show virtually no specific affinity with the dextran polymer, and release was expected to be solely by diffusion. The CD (DMF) 1:0.16, CD (DMSO) 1:0.16, and CD (DMSO) 1:0.32 polymers had very similar release patterns. MMC release from all of these polymers began to plateau near 50–75 h, which indicates a release mechanism in addition to that of diffusion alone. The CD (DMF) 1:0.32 polymers had the most prolonged release rate, as shown by a nearly linear release during the first 12.5 h. Although these polymers did not swell as much during the swelling study, and the SEM images indicated rigidity, the release rate from these materials was the most prolonged out of all polymer formulations. The CD (DMF) 1:0.32 polymers had a slower MMC release compared to the CD (DMF) 1:0.16 polymers because a higher crosslink ratio tends to reduce solvent swelling, which impacts drug release and even drug loading. A possible explanation for the release is that when using DMF to make CD disks, the CD is able to pack more tightly, and form more crosslinks between CD molecules than when made with DMSO.

Additionally, we showed that both dextran disks, and CD disks made with DMSO, had higher swelling ratios than CD disks made with DMF. These materials were also shown by SEM to have macroscopic porosity. This higher swelling and presence of porosity, possibly show more rapid loading into the center of a device, but also show more rapid release from that device. A drug molecule in the center of a DMSO-made material can hop out of a CD pocket, into a water filled channel, and rapidly diffuse out of the device. While another drug molecule in the center of a DMF-made material after hopping out of one CD pocket, doesn't

have an easy channel and must diffuse through the bulk of the polymer, potentially falling back into another CD pocket. Inclusion complex formation was indirectly confirmed by drug loading experiments. The results in Table 2 show very similar amounts of total MMC loaded into CD disks regardless of solvent and extent of crosslinking (0.17–0.19 μg), while the dextran disks nearly 75% less MMC (0.04–0.50 μg).

As shown in the results section, effective inhibition of fibroblast proliferation, from released MMC, indicates that minimal amounts of MMC are needed to diminish scar tissue in this model using low cell counts. Also, the fact that fibroblast cell counts either remained the same, or increased, during the MTS assay suggests that there was generally low to no gross toxicity of the MMC released from the polymers. Released MMC from dextran polymers became insufficient to prevent fibroblast proliferation much more quickly than MMC release aliquots from the CD polymers. This is supported by the individual aliquot release data, which shows most of the MMC is released from the dextran polymers at early time points and rapidly falls below effective delivery concentrations, as compared to the slower, sustained release from the affinity-based CD polymers.

5. Conclusions

The data indicates that MMC maintains efficacy as an anti-proliferative drug after incorporation and release from an affinity-based polymer system. The CD polymers show a more prolonged *in vitro* release rate of MMC, than a comparable diffusion-only system. Additionally, cell proliferation assays using aliquots of MMC show an IC_{50} of approximately 0.4 $\mu\text{g/mL}$. Seeing that no more than 0.2 μg was released from any individual gel, while still maintaining extended therapeutic effect, suggests that this therapy will present less clinical risk to both the patient and surgical staff, due to less overall exposure to MMC than more traditional treatments. The first 100 h of delivery, CD polymers were releasing within this therapeutic dosage range, and effectively inhibited fibroblast cell proliferation. Additionally, seeing that the solution half-life of MMC has been reported to be between 11 and 27 min (Kawase et al., 1992), the maintained drug efficacy after 100 h of delivery suggests that the CD polymer provides a stabilizing or protecting environment preventing MMC degradation. The use of CD polymers to release MMC after glaucoma filtration surgeries is likely improve surgery success rates, reducing the need for additional intervention due to excess scar tissue formation. In this study we did not specifically evaluate biodegradability. It is possible that it shows long-term degradability as cyclodextrin is made of α 1–4 glycosidic linkages which human enzymes are capable of breaking down. In the short-term of this study no overt degradation was detected. Cyclodextrin by itself is generally regarded as safe and has been used in a number of different FDA approved formulations. Insoluble polymer of cyclodextrin has been shown by us and by others to have no detectable cellular toxicity. These factors together combine to indicate that this polymer platform a viable technology for ophthalmologic delivery of MMC. Additionally, the potential for this polymer platform to be molded into a contact lens (Xu et al., 2010) would allow for easy administration of MMC, or delivery other ocular therapeutics.

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Abbreviations

MMC	mitomycin C
CD	cyclodextrin
IOP	intraocular pressure

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Fig. 1.
Experimental schematic of the approach for MMC drug release studies.

Fig. 2. Low magnification view of polymer disks in a 24-well tissue culture plate. Thin films are cast from polymers, allowed to crosslink, and then disks are punched using an 8 mm punch.

Fig. 3. Scanning Electron Microscope images of the polymers. A) Cyclodextrin gel made in DMSO, B) Cyclodextrin gel made in DMF, C) Dextran gel.

Fig. 4.

A: Polymer swelling in water and DMF. While water is most similar to the type of release media we use in the study, swelling in DMF gives information on maximal swelling capabilities of polymers made in DMF. B: Polymer swelling in water and DMSO. Compared to Fig. 3A, the polymers made in DMSO appear to swell more than twice as much, in organic solvent and in water, than polymers made in DMF.

Fig. 5. Cumulative drug release from six different types of polymers over a 172-h period. Experiments were done in triplicate ($n = 3$). Error bars represent $\pm$ standard deviation. Delivery appears to have completed at approximately 10 h for the dextran disks, and 100 h for all CD disks. The standard deviation bars show minimal dispersion from the mean values of released drug concentrations.

Fig. 6.

Individual mass of released drug at each time point. Experiments were done in triplicates ($n = 3$) and error bars represent $\pm$ standard deviation. This is data from Fig. 4 re-plotted in a different semi-log representation, showing individual amounts of drug released at each time point. In both plots, one can see at least 1–2 orders of magnitude difference in amounts of MMC released, where the CD disks release much more MMC than dextran disks at each time point.

Fig. 7.

A: Cell proliferation assay using NIH 3T3 cells incubated with released MMC from CD polymers made with a 1:0.16 crosslink ratio. In this plot, the diffusion-only release from dextran begins to lose the ability to inhibit proliferation at approximately 12 h. The CD affinity-based polymers are able to inhibit proliferation for at least 75 h. B: Cell proliferation assay using NIH 3T3 cells incubated with released MMC from CD polymers made with a 1:0.32 crosslink ratio. In this plot, the diffusion-only release from dextran begins to lose the ability to inhibit proliferation at approximately 12 h. The CD affinity-based polymers are able to inhibit proliferation for at least 95 h.

Fig. 8. MMC anti-proliferation curve using known concentrations of MMC. This curve indicates the IC₅₀ value of MMC in this system is in the $\mu\text{g/mL}$ range. The IC₅₀ is three orders of magnitude lower than the loading concentration, and gives a target delivery range for an effective dosage with low toxicity.

Table 1

Comparison of polymer compositions.

Polymer	Crosslinking molar ratio (glucose unit: HDI)	Weight of polymer (g)	Solvent used during synthesis	Amount of solvent (mL)
Gamma-CD	1:0.16	1	Dimethylformamide	4
Gamma-CD	1:0.32	1	Dimethylformamide	4
Gamma-CD	1:0.16	1	Dimethyl sulfoxide	4
Gamma-CD	1:0.32	1	Dimethyl sulfoxide	4
Dextran	1:0.16	1	Dimethyl sulfoxide	4
Dextran	1:0.32	1	Dimethyl sulfoxide	4

Table 2

Total drug loaded into each polymer.

Polymer formulation	Average (µg)	Standard deviation (µg)
Gamma CD DMF 1:0.16	0.188	0.016
Gamma CD DMF 1:0.32	0.174	0.012
Gamma CD DMSO 1:0.16	0.192	0.018
Gamma CD DMSO 1:0.32	0.181	0.016
Dextran DMSO 1:0.16	0.052	0.009
Dextran DMSO 1:0.32	0.047	0.008