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Enzymatic Degradation of a γ-Cyclodextrin-Hyaluronic Acid Copolymer

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Abstract: Postoperative scarring following trabeculectomy, a glaucoma surgery, often leads to failure, necessitating biodegradable drug delivery systems for sustained antifibrotic release. This study explores γ-cyclodextrin/hyaluronic acid (γ-CD/HA) copolymers as potential drug depots, evaluating their degradation kinetics under physiological conditions. Copolymers with 0% and 75% γ-CD were synthesized via base-catalyzed crosslinking with 1,4-butanediol diglycidyl ether (BDDE). Degradation was assessed by incubating samples in Sørensen buffer (pH 7.4) with lysozyme, monitoring mass loss over 14 days. Both systems degraded, yet the 75% CD copolymer exhibited a significantly slower, linear degradation profile compared to the rapidly degrading 0% CD system. Morphological analysis revealed increased swelling in the 75% CD samples, suggesting cleavage of intermolecular crosslinks. Gravimetric analysis indicated a mass loss of approximately 50% for the 75% CD system by day 14. The tunable degradation kinetics of these γ-CD/HA copolymers suggest promise for controlled drug release applications in glaucoma, warranting further investigation into drug loading and release profiles.

Keywords: hydrogel degradation, Local Drug Delivery, Glaucoma treatment, trabeculectomy

1 Introduction

1.1 Glaucoma

Glaucoma is a group of eye conditions characterized by progressive damage to the optic nerve, often associated with elevated intraocular pressure (IOP). This chronic disease is one of the leading causes of irreversible blindness worldwide [1]. The most common form, open-angle glaucoma, typically develops slowly and asymptomatically, making early detection and treatment crucial for preserving vision.

The primary goal of glaucoma treatment is to reduce IOP to prevent further optic nerve damage. Initial management often involves topical medications, such as prostaglandin analogs, beta-blockers, or carbonic anhydrase inhibitors. When pharmacological interventions prove insufficient, laser treatments or surgical procedures may be necessary. Among surgical options, trabeculectomy has long been considered the gold standard for IOP reduction in advanced cases.

While trabeculectomy is effective in lowering IOP, it is associated with significant risks and complications. The procedure creates an artificial drainage pathway for aqueous humor, but postoperative scarring can lead to failure in up to 50% of cases within one year [2]. Complications may include hypotony, choroidal effusion, and infection [3]. The use of cytostatic agents like mitomycin C can reduce scarring but increases the risk of other complications.

Recent advancements in drug delivery systems have shown promise in addressing the limitations of trabeculectomy. We developed an antifibrotic drug delivery system that has been tested in animal models [5]. This approach aims to modulate wound healing and reduce scarring at the surgical site, potentially improving long-term outcomes of glaucoma filtration surgery. The reported antifibrotic drug delivery system was non-degradable, necessitating additional procedures for removal. This limitation has sparked interest in developing biodegradable alternatives that can deliver therapeutic agents effectively and then safely degrade over time, eliminating the need for removal surgeries.

Cyclodextrins (CDs) and hyaluronic acid (HA) have emerged as promising materials for degradable drug delivery [6]. CDs possess the ability to form non-covalent inclusion complexes with various drug molecules, enhancing their solubility and stability [4]. HA, a natural component of the eye, is known for its biocompatibility and is already widely used in ophthalmic applications. The combination of these materials offers potential for creating biocompatible, degradable drug delivery systems for glaucoma treatment [7].

This study aims to investigate the coupling of γ-CD with HA and evaluate its degradation profile. The ultimate goal is to develop a biodegradable drug delivery system capable of

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releasing antifibrotic agents during trabeculectomy. Such a system could potentially improve surgical outcomes while eliminating the need for revision surgeries to remove non-degradable implants, thus advancing the field of glaucoma management.

2 Materials and Methods

2.1 Materials

γ-Cyclodextrin was purchased from Wacker Chemie AG (MW: 1297.12 g/mol, 98%, Germany). 1,4-butanediol diglycidyl ether (BDDE) and hyaluronic acid sodium salt (MW: 1.5-1.8x10^6 Da) was purchased from Sigma-Aldrich (Germany).

2.2 Synthesis of γ-CD/HA-Copolymer

The synthesis of γ-cyclodextrin/hyaluronic acid (γ-CD/HA) co-polymers was achieved through base-catalyzed cross-linking with 1,4-butanediol diglycidyl ether (BDDE). In accordance with the target specifications, HA (hyaluronic acid) and γ-CD were weighed in mass fractions of 0% and 75% (total mass: 0.5 g) and suspended in 5 mL of 0.001 M NaOH solution (pH 11). The alkaline condition served to open the HA helices and activate the hydroxyl groups for the cross-linking reaction. Homogenization of the resulting opaque suspension was performed by repeated centrifuge mixing for 90 min (Hausschild Speedmixer, Hamm, Germany) and manual disintegration of agglomerates using a spatula. Subsequently, 400 µL BDDE was added and homogenized over 90 min with further centrifuge mixing. The viscous mass was cast into PTFE molds (ø = 2.5 cm, h = 1 mm) and air-dried at room temperature for two days to obtain flexible films.

2.3 Copolymer Degradation Study

Degradation studies were performed as reported earlier [8], samples (wet-punched, dried, and weighed) were incubated in Sørensen buffer (pH 7.4) containing 1.5 µg/mL lysozyme. The buffer was changed daily to maintain enzymatic activity. Degradation was monitored over two weeks at several time points using gravimetric mass loss analysis. The combination of hydrolytic (pH 7.4) and enzymatic lysozyme-mediated cleavage simulated physiological conditions.

Table 1: Timepoints and sample counts.

Time point (d)	0	2	4	6	7	8	11	12	14
Sample Count 0% CD	3	3	2		3				
Sample Count 75% CD	3	3	3	3		3	3	2	3

One sample at day 4 and 12, respectively was lost during the medium change, thus only two samples remained for analyses. Sample masses were determined using a KERN 770-33 analytical balance (KERN & SOHN GmbH, Germany). Prior to imaging, samples were fully swollen in water to ensure maximal hydration of the polymer network.

3 Results and Discussion

3.1 Gravimetry

All samples showed a decreasing mass during the tested time period (figure 1). As expected, this was more pronounced for the 0% CD. The samples exhibited progressive mass loss attributable to the degradation of the HA matrix via breakdown of the β-1,4-glycosidic bonds, which are known to be cleaved by lysozyme. Due to the intense degradation, the samples could not be handled anymore after day 7. Therefore, degradation was stopped for the 0% CD samples.

In contrast to this, the 75% CD samples showed mass loss until the end of study at day 14, where it resulted in around 50%. High standard deviations are interpreted as hints towards inhomogeneities of the samples.

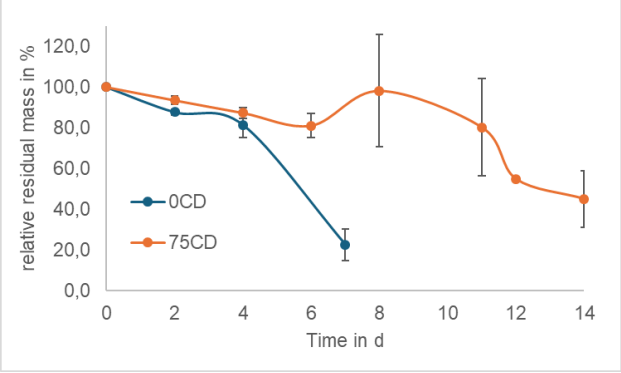

Figure 1: Blue graph shows the mass loss of 0% CD over 7 days and orange graph the mass loss of around 50% of 75% CD within 14 days

Although the degradation appeared approximately linear, it proceeded more slowly for the 75% CD samples compared to the 0% CD samples. This suggests that mass loss was not solely attributable to the removal of HA residues, which would theoretically cease after 75% mass reduction. The continued decrease in mass indicates the subsequent diffusion of CD molecules or their fragments from the bulk material.

3.2 Morphological characterisation

In figure 2 macrographs of the hydrogels are shown. The degradation of 0% CD gels revealed a massive change in morphology from a disc shape at day 0 to an irregular shape at day 7. The degradation of the 75% CD samples revealed strong swelling after 14 days, probably due to the cleavage of intermolecular crosslinks and thus by forming greater pores that are able to bind additional water molecules. However, the disc shape still remained until the end of this study.

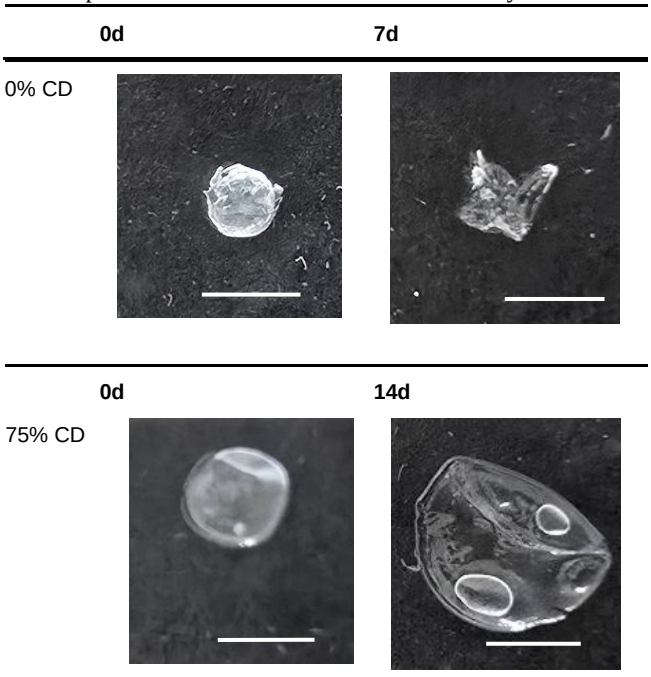

Figure 2: Representative pictures of the hydrogels. For the 0% CD gels at day 0 and day 7 (top row), the degradation also led to a change in morphology from a circular to an irregular shape. For the 75% CD samples at day 0 and day 14 (bottom row), degradation led to increased swelling after 14 days. Bars represent 5 mm.

4 Conclusion

γ-CD/HA copolymers demonstrate tunable degradation kinetics. Both systems (0% and 75% CD) degraded within the study presented here under physiological conditions. The 75% CD variant degraded 2.4×times slower, showing linear mass loss versus the degradation measured from CD-free samples. This predictable degradation almost aligns with trabeculectomy's 7–14 day antifibrotic treatment window. CD-enhanced crosslinking and hydrophobic interactions retard hydrolysis while maintaining biodegradability. Future studies will optimize drug (e.g., Josamycin [5, 9, 10]) loading/release profiles using CD-host-guest chemistry. These systems promise self-eliminating implants to reduce revision surgeries in glaucoma management.

Author Statement

Use of AI: This text incorporates content generated by an AI assistant (https://www.perplexity.ai/) to enhance clarity and conciseness. While the authors take full responsibility for the scientific integrity of this publication. Acknowledgements: The authors would like to thank Caroline Dudda for their excellent technical assistance. Conflict of interest: Authors state no conflict of interest

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