HYALURONAN-CONJUGATES FOR CONTROLLED OCULAR DRUG DELIVERY

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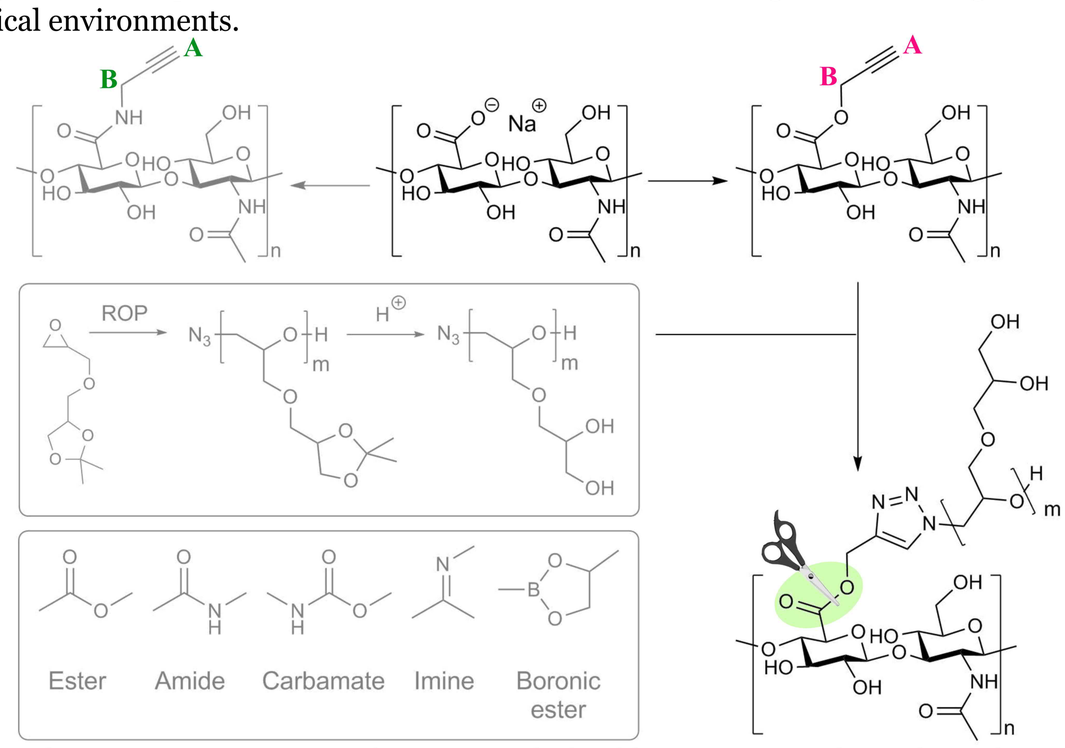
Introduction

Retinal diseases are a leading cause of visual impairment in the aging societies. They are often caused by extensive blood vessel growth and blood leakage from the choroid, which can be treated with anti-angiogenic drugs (such as dexamethasone). Small molecular weight drugs have very short half-lives in the vitreous (<10 h), rendering their dosing intervals too short to be acceptable for intravitreal injection. Controlled release delivery systems can lower the amount of necessary injections and deliver drugs over a prolonged period of time.

We propose an entirely water-soluble graft copolymer as a platform to release small drugs via a two-stage mechanism (Scheme 1). The copolymer is designed to achieve long retention times and sustained release in the vitreous by means of high molecular weight and slowly cleavable bonds. Here we present the synthesis of the graft copolymer and methods to quantify the polymer-from-polymer release, a topic that is rarely covered in the literature.

Synthesis & Properties of the Graft Copolymer

Hyaluronic acid (HA) serves as backbone of the graft copolymer and is functionalized with hydrolysable linkers.[1] Poly(glyceryl glycerol) (PGG) side chains, prepared by ring-opening polymerization,[2] are grafted to HA via click reaction. The hydroxyl moieties in every PGG repeating unit can be used to attach drugs, probes and targeting ligands. Various degradable linkages are incorporated to enable the selective release of grafts and drugs in different chemical environments.



Scheme 2. From top to bottom: Hydrolysable HA-propargyl amide and ester derivatives. Synthesis of PGG and click-grafting to HA. Degradable linkages for selective release.

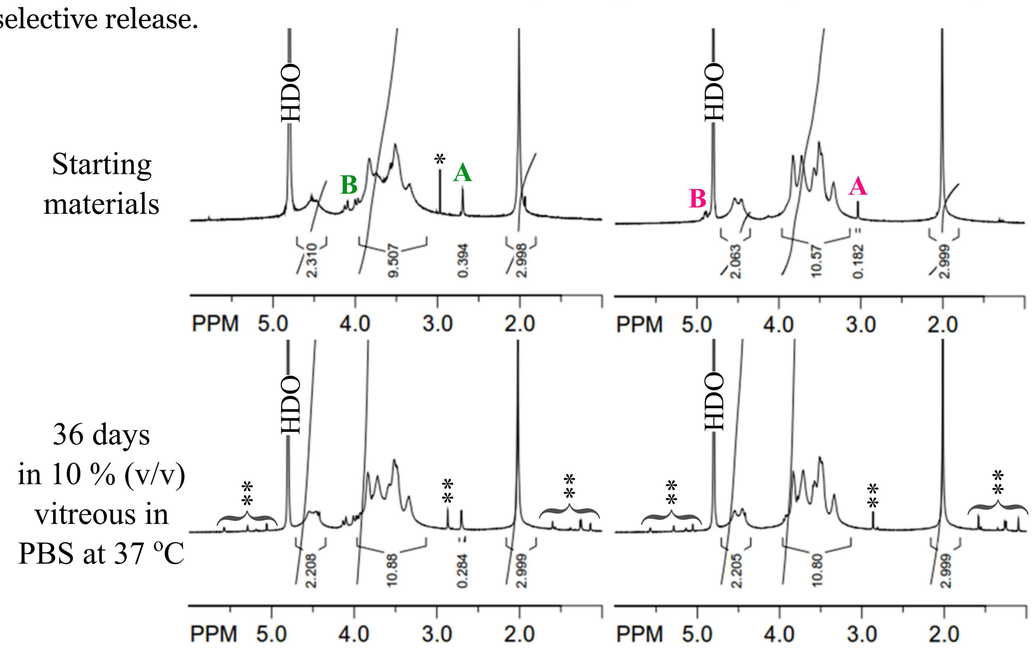


Figure 1. Cleavage of the hydrolysable bonds was investigated by NMR. While HA-esters were hydrolyzed completely within 36 days, amide peaks decreased by 28 %.

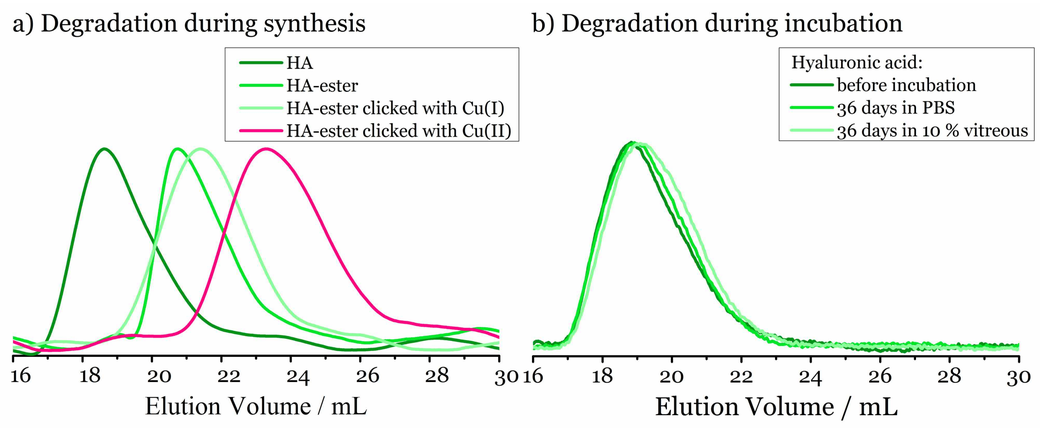
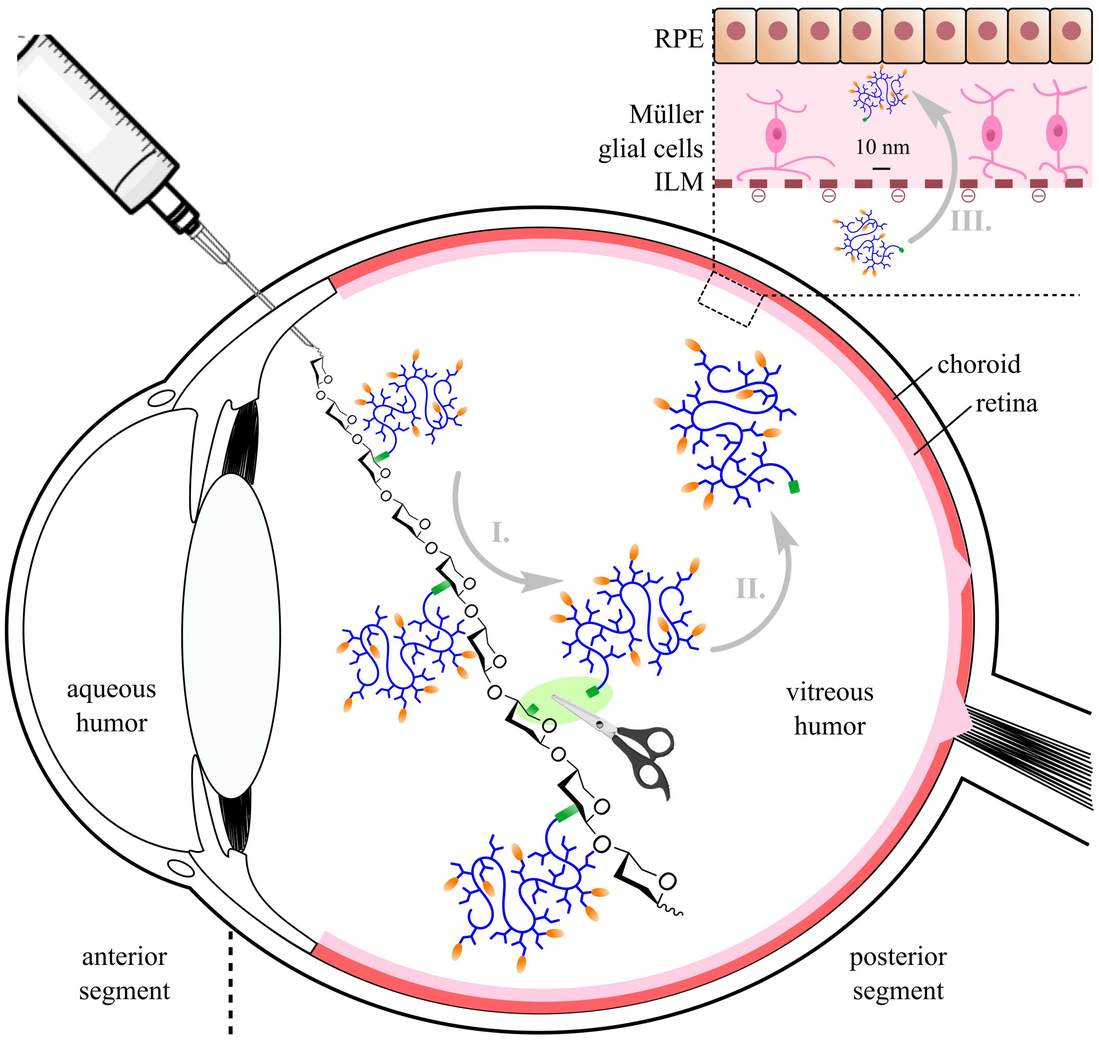


Figure 2. Molecular weight changes of HA were followed with SEC after: a) chemical modification and b) incubation. Procedures were chosen to minimize HA degradation.



Scheme 1. Design and mechanism of action of the drug delivery system: I. After intravitreal injection, the graft copolymer slowly releases its drug-carrying side chains (stage 1). II. The side chains freely diffuse in the vitreous towards the retina. III. They can overcome the barriers of the inner-limiting membrane (ILM) and reach the retinal pigment epithelium (RPE) to deliver their cargo (stage 2).

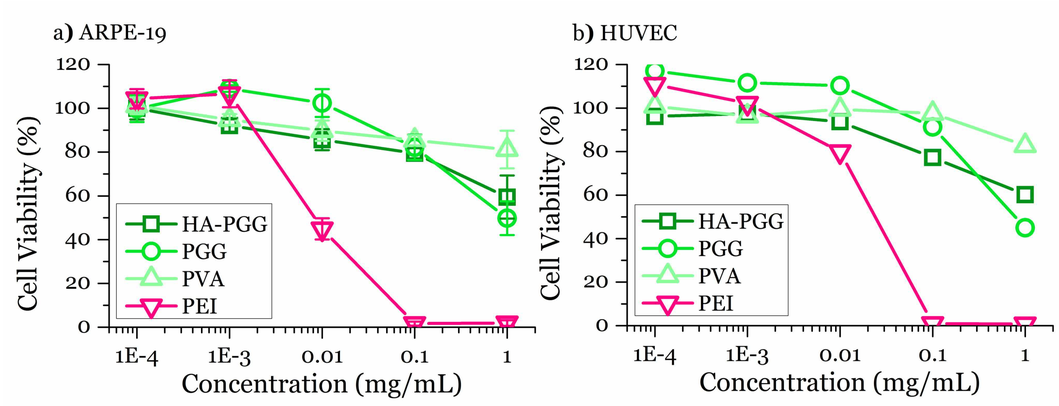
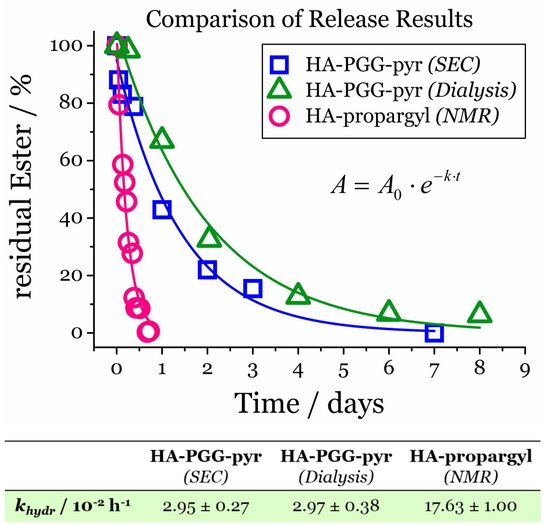


Figure 3. Biocompatibility of HA-PGG and released PGG towards a) human retinal pigment epithelium (ARPE-19) and b) human umbilical vein endothelial cells (HUVEC) determined by MTT cytotoxicity assay. FDA-approved polyvinyl alcohol (PVA) and toxic polyethylene imine (PEI) were measured for comparison.



	HA-PGG-pyr (SEC)	HA-PGG-pyr (Dialysis)	HA-propargyl (NMR)
k_{hydr} / 10^{-2} h^{-1}	2.95 ± 0.27	2.97 ± 0.38	17.63 ± 1.00
k_{diff} / 10^{-2} h^{-1}	-	10.50 ± 3.31	-
$t_{1/2}$ / h	23.51 ± 2.19	23.34 ± 3.04	3.93 ± 0.22
adj. R^2	0.986	0.997	0.970

Figure 4. Release of pyrene-labeled PGG from the HA graft copolymer was studied by dialysis and SEC. Dialysis data was fitted with a 2-step model considering hydrolysis and diffusion rates. Release of propargyl alcohol from the HA-ester was studied by NMR. Hydrolysis rate constants (k_{hydr}) and half-lives ($t_{1/2}$) of the derivatives are summarized in the table.

Conclusion & Outlook

HA-PGG graft copolymers with ester linkages were synthesized and release of the grafts was studied. The HA-backbone was stable during the experiments. Release of the PGG side chains took place via ester hydrolysis over the course of one week. Dialysis and SEC were used to estimate the hydrolysis kinetics and the results of both methods corresponded well with each other. The release of polymeric side chains was found to be slower than that of small molecules, probably due to steric effects. Both, the HA-PGG graft copolymer and released PGG, were biocompatible up to 0.1 mg/mL, which is the anticipated concentration after intravitreal injection.

Next, the hydroxyl groups of PGG will be functionalized with targeting ligands to enhance the cell uptake of the grafts. Drugs will be attached via selected degradable linkages to achieve intracellular drug release from PGG (stage 2).

Publications:

[1] T. Borke, F.M. Winnik, H. Tenhu, S. Hietala, *Carbohydr. Polym.* 116 **(2015)** 42–50. doi:10.1016/j.carbpol.2014.04.012.

[2] T. Borke, A. Korpi, F. Pooch, H. Tenhu, S. Hietala, *J. Polym. Sci. Part A Polym. Chem.* **(2017)**. doi:10.1002/pola.28497.

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