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PEPTIDE ANTIBODY CONJUGATES TO TREAT NEOVASCULAR AGE-RELATED MACULAR DEGENERATION

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Introduction: Age-Related Macular Degeneration (AMD) is a chronic disease of the macula of the eye (Figure 1) and leading cause of blindness in Americans over 50 [1]. Neovascular type AMD (nAMD) is the less common yet more severe form of the disease and is characterized by proliferation of abnormal, leaky blood vessels in the choroid (neovascularization) (Figure 1) [2]. This blood vessel growth is stimulated by an overexpression of signaling molecule Vascular Endothelial Growth Factor (VEGF) and is responsible for cell death that causes blindness in the disease state [2], [3]. NAMD is often treated with agents that inhibit VEGF such as ranibizumab [4]. Ranibizumab is an anti-VEGF antibody fragment (fab) that binds to and deactivates VEGF and is over 90% effective at preventing progressing blindness in nAMD patients [4]. The fab is administered through intravitreal injections, which occur frequently due to rapid clearance of the fab from the eye ($t_{1/2}$ ~ 9 days) [4], [5]. The uncomfortable nature of these injections combined with their frequency (every 1.5 months on average) [6] contribute to high patient burden and subsequent low treatment compliance [5]. There is a need to increase adherence of the fab to the vitreous; thus, this project looks to binding peptides to target and anchor the fab to vitreal components.

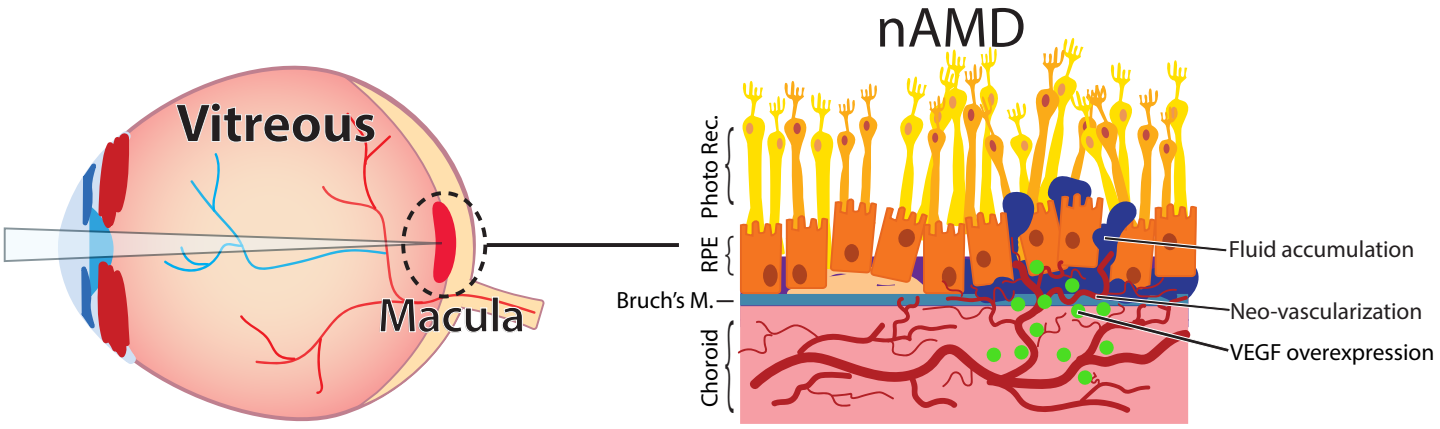

Figure 1 Simplified image portraying key anatomy of the eye along with features that characterize the nAMD disease state.

Project Aim: The aim of this project is to extend the intraocular half-life of ranibizumab by attaching ECM targeting binding peptides with the goal of anchoring the fab. The ultimate goal is to decrease patient treatment burden by decreasing the frequency and increasing time between patients' injections. We chose two abundant molecules in the eye's vitreous—hyaluronic acid (HA) and type II collagen (col)—as our binding peptide targets and synthesized the binding peptides based on established, literature-referenced peptide sequences.

Methods: A col binding peptide (CBP) and HA binding peptide (HABP) based on sequences from Rothenfluh, et al. [7] and Turley, et al. [8], respectively, were synthesized to target type II collagen and HA. The CBP and HABP were synthesized via solid phase peptide synthesis and purified via high performance liquid chromatography. The peptides were conjugated to the ranibizumab fab via a maleimide thiol click reaction; a simplified reaction scheme is detailed in Figure 2. After conjugation, the conjugates were purified and characterized by mass spectrometry. The conjugates' binding affinity for their respective target molecules was tested using ELISA-like assays (Enzyme Linked Immunosorbent Assay). Binding of the conjugates to VEGF was also tested to determine whether VEGF-binding is negatively impacted by peptide conjugation.

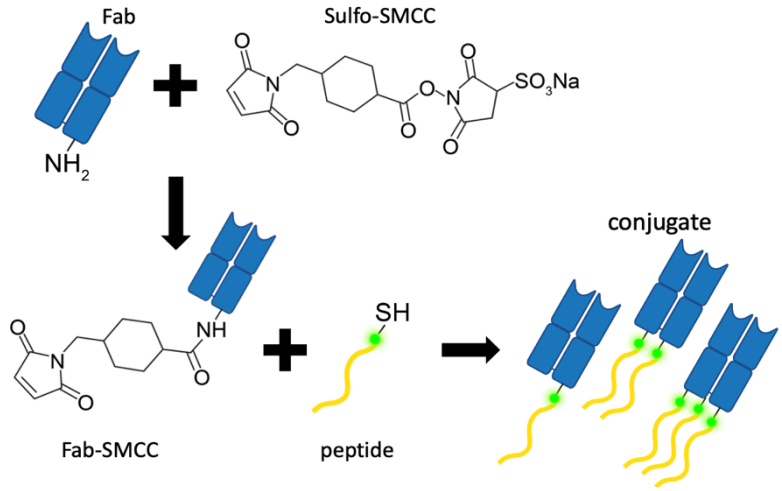

Figure 2 Simplified conjugation reaction sequence used to conjugate binding peptides to fab. First, a sulfo-SMCC linker group was attached to the fab at lysine amino acids. Second, the peptide was attached by reacting the thiol group on the peptides' cysteine amino acids with the maleimide on the fab-SMCC.

Results: Mass spectrometry after conjugation of the peptide to the fab revealed multiple peptides bound per fab. For the CBP-fab conjugate, an average of 1.1 CBPs were bound per fab; an average of 4.3 HABPs were bound per fab for the HABP-fab conjugate.
HA and col were immobilized to wells of a 96-well plate for the first ELISA-like assay (details depicted in Figure 3). The HABP-fab conjugate and CBP-fab conjugate bound in much higher quantities to their target molecules compared to unmodified fab controls with high statistical significance (Figure 4).

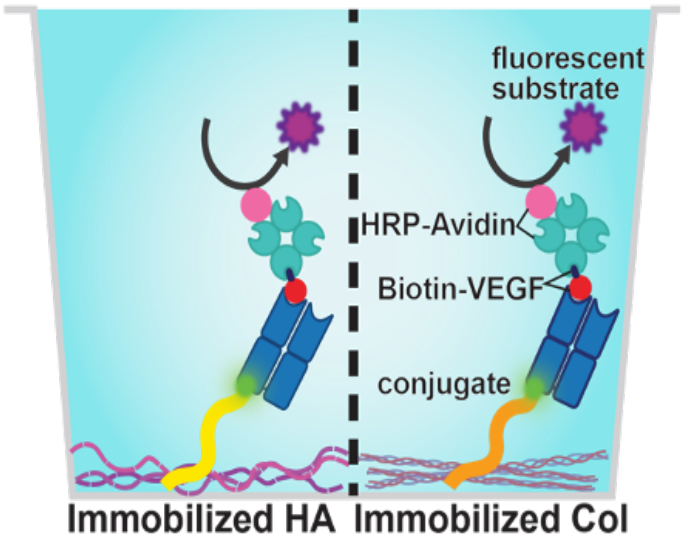

Figure 4 ELISA binding scheme for immobilized HA and col binding assay. First, HA or col was immobilized to the well, then the conjugate was added. The conjugate simultaneously binds to the target HA or col and biotin-bound VEGF. HRP-Avidin subsequently binds biotin, and HRP undergoes a reaction to produce a fluorescent substrate, which is read by a plate reader for comparison.

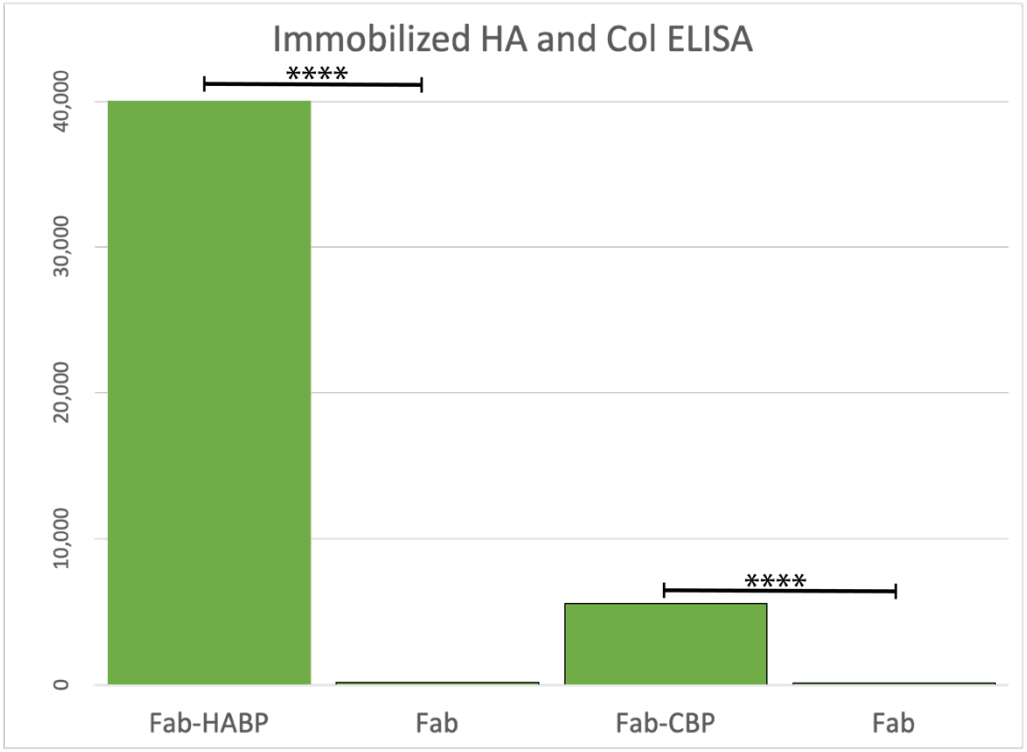

*Figure 3 Fluorescent signal readings from immobilized collagen and immobilized HA ELISA assay. Vertical axis displays unitless relative fluorescence units (RFU). Fab-HABP bound in much higher quantities to HA compared to unmodified fab. The fab-CBP conjugate bound in much higher quantities to col compared to the unmodified fab. Statistical significance is defined as follows: ns = P > 0.05; * P ≤ 0.05; ** P ≤ 0.01; ** P ≤ 0.001; *** P ≤ 0.0001.*

For the second ELISA assay, VEGF was immobilized to wells of a 96-well plate and the conjugates were added followed by substrates to amplify their binding as outlined in Figure 5. The results for this VEGF binding test are displayed in Figure 6 and show equivalent binding of both conjugates to VEGF compared to the unmodified fab.

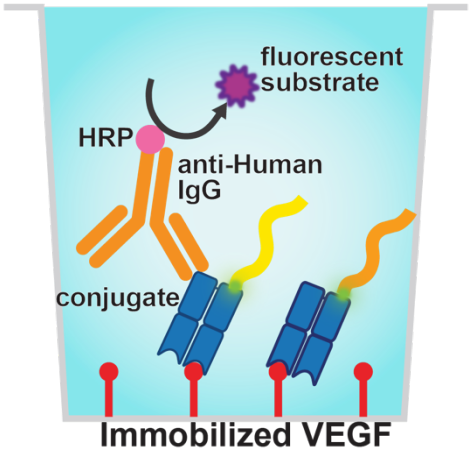

Figure 6 ELISA binding scheme for immobilized VEGF binding test. VEGF immobilized to the plate and conjugates were added to bind. Binding of conjugates was amplified by addition of HRP bound anti-Human IgG to target the fab, followed by fluorescent substrate producing reaction using HRP. Fluorescent signa was read by a plate reader for comparison.

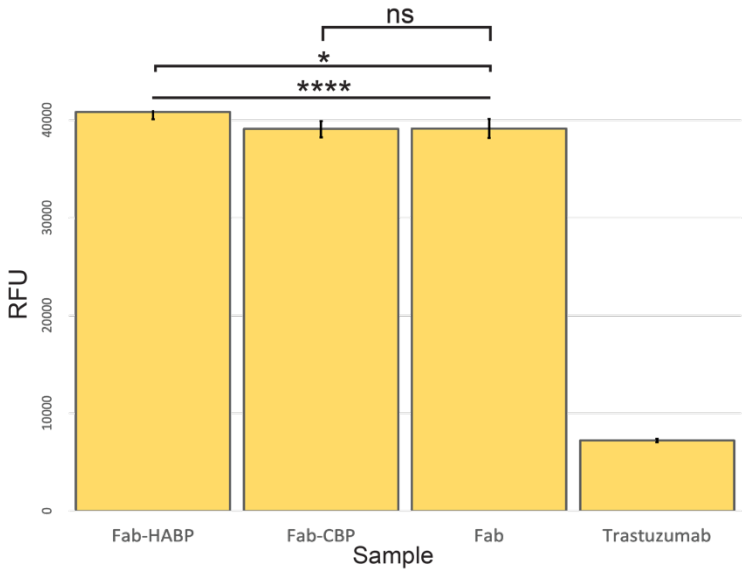

*Figure 5 Results for VEGF ELISA. Fab-HABP and fab-CBP conjugates show similar binding to VEGF compared to unmodified fab. All fab-containing compounds (first three columns) bind significantly higher than trastuzumab, a non-VEGF binding antibody and negative control in this experiment. Statistical significance is defined as follows: ns = P > 0.05; * P ≤ 0.05; ** P ≤ 0.01; ** P ≤ 0.001; *** P ≤ 0.0001.*

Conclusion: Both fab-CBP and fab-HABP conjugates bound to their target molecules col and HA, respectively, in significantly higher quantities compared to the unmodified fab. Furthermore, the conjugates bound to VEGF as well as the unmodified fab. The combination of the conjugates' ability to bind to VEGF along with their significantly improved affinity for HA and col leads us to expect the peptides will slow clearance of ranibizumab from the eye's vitreous post injection.

References:

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