Diagnostic AβO-targeted probes for Alzheimer's Disease

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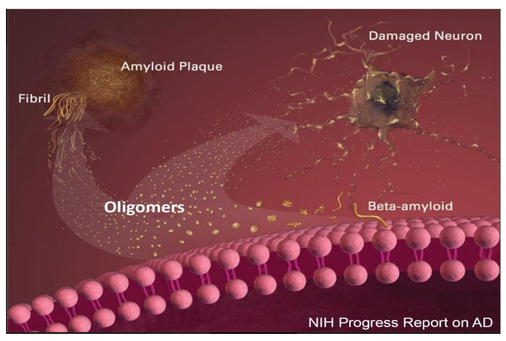
Abstract

Using a double conjugation method, we developed a probe with high AβO-specific antibody and magnetic nanoparticle conjugation efficiency. This probe was successfully used in mouse and rabbit models to detect the presence of toxic AβOs in Alzheimer's disease and image AβO clusters in MRI imaging.

Introduction

Aβ Oligomers (called AβOs) are small peptides that show early build-up in immunohistochemistry and immunochemistry studies, and appear before plaques in Alzheimer's development. High levels of AβOs cause loss of memory performance and other pathological changes.Early presentation of AβOs makes them ideal for diagnostic targeting with AβO-specific antibodies Bound to magnetic nanostructures (MNSs) for MRI visibility. Further testing can determine effectiveness of future pharmaceuticals for AD therapy.



Materials and Methods

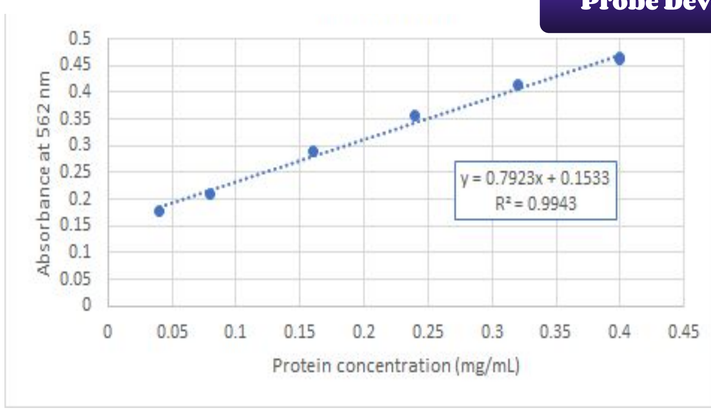
MRI Probe Conjugation: The materials required for this are MNS (magnetic nanostructures), ACUMNS Antibody, Coupling Buffer, and Activation buffer. First, the MNS is bound to ACU193 using coupling buffer and activation buffer, which causes S-NHS to bind the receptors on the two entities. Once the conjugation is complete we use the BCA Assay to check the percent conjugation on the ACUMNS.

BCA Assay: This simple method that uses the supernatants from ACU193 containing unbound antibody and passes them through an absorbance machine at 562 nm to indicate how much antibody was not bound to the MNS.

Immunohistology on Mouse and Rabbits Brain Slices: In this process, a secondary antibody is bound to an immunofluorescence tag which indicates the presence of amyloid beta. Brain slices from mouse and rabbit samples are washed with this solution and later imaged in a microscope to observe signs of lingering amyloid-beta.

Results

Probe Development



	Absorbance of ACUMNS Solution		
	Original ACU193 Sample	After 1 Conjugation Flow-Through	After 2 Conjugation Flow-Throughs
Trial 1	0.111	0.110	1.863
Trial 2	0.107	0.107	1.869
Average	0.109	0.1085	1.866
Protein Concentration (mg/mL)	-0.054	-0.054	2.134

Figure 1 (left): This is the absorbance graph was made from the data provided by the BCA Assay of standard concentrations. The line of best fit from this data was used to determine the protein concentration of supernatant and ACUMNS samples in the double conjugation method.

Figure 2 (right): This table on the right demonstrates a source of error, due to an underestimation of our line of best fit, but still shows progression from the the original sample probes to the new probes, which has a conjugation efficiency of 71.3%.

Mouse Imaging

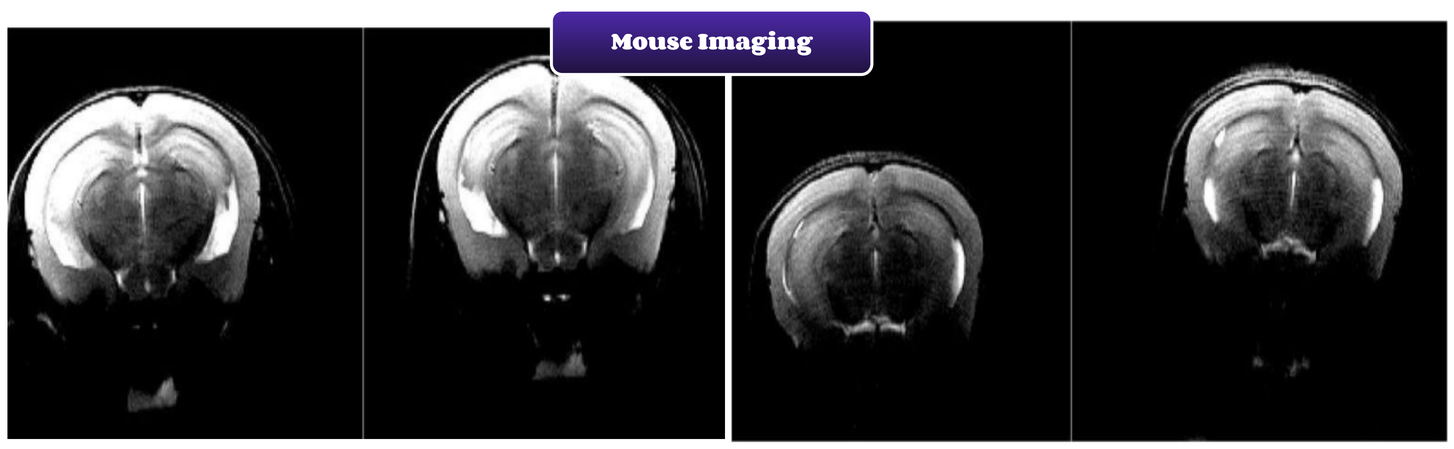


Figure 3 (left) & 4 (right): This is one of our early mouse MRI scans and depicts the presence of probe. The figure to the left is a wild type, thus we should see no presence of the probe, while the figure on the right is a transgenic mice model. The darker gray areas indicate the probe signaling.

Rabbit Imaging

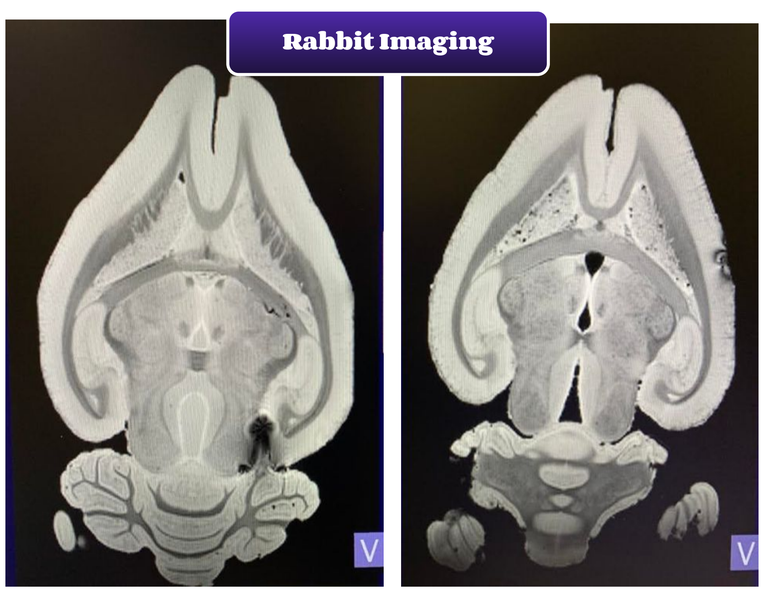


Figure 5: This is an example of an ex vivo rabbit image. The image on the left is control (pre-injection) and the right is post-injection. Dark spots in hippocampal region at the top on right indicate probe signaling.

Conclusion & Discussion

Using the new double conjugation method, we were able to improve the conjugation efficiency of protein to MNS to 71.3%. This new probe was used in the rabbit model, and has been successful in preliminary tests. The presence of dark black spots in figure 5 (top of right image) above demonstrate the AβO-specific antibody binding to AβOs in the hippocampal region and the MNS bound to these antibodies giving off a strong signal in the MRI scan images. This signal did not significantly decrease after 48 hours, indicating long-term viability of the probe. In the future, we will work on testing the probe on a larger rabbit population to confirm our preliminary results, and begin to work on developing a probe for human use. The rabbit models proved to be more effective than the mice models, and the results from this series of experiments are more significant due to the rabbit's 99.9 homology with humans for their AD gene. The mice's successful tests provided a segway into the imaging of the rabbits, allowing us to realize that the probes effectively detected amyloid-beta oligomers. This diagnostic model has the potential to improve the efficiency of Alzheimer's diagnostics, saving energy, people, and money on a global scale, as well as improve pharmaceuticals by determining the true impact these future drugs will have on onset.

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