
**Selective activation of endothelial monolayers
by soluble aggregates of the amyloid- β protein:
Potential for exploitation in a cell-based sensor**

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Alzheimer's Pathology

Alzheimer's Brain

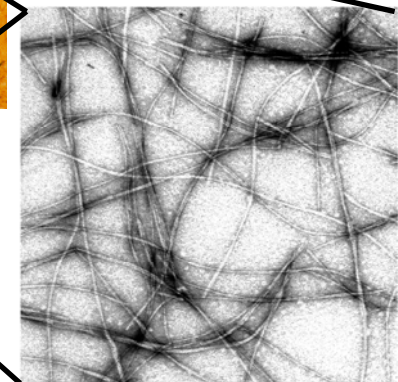
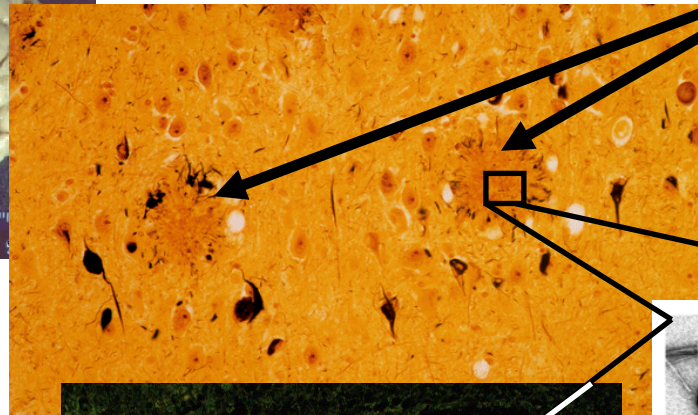


Courtesy of D. Dickson, Mayo Clinic, Jacksonville.

Pathology

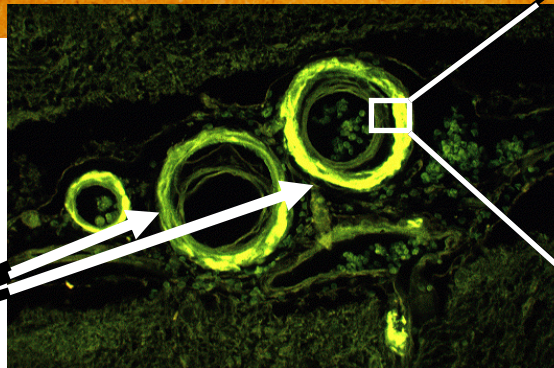
Amyloid Plaques

A β Fibrils



Nichols *et al.* (2002) *Biochemistry*, 41: 6115-27.

Cerebral Amyloid Angiopathy

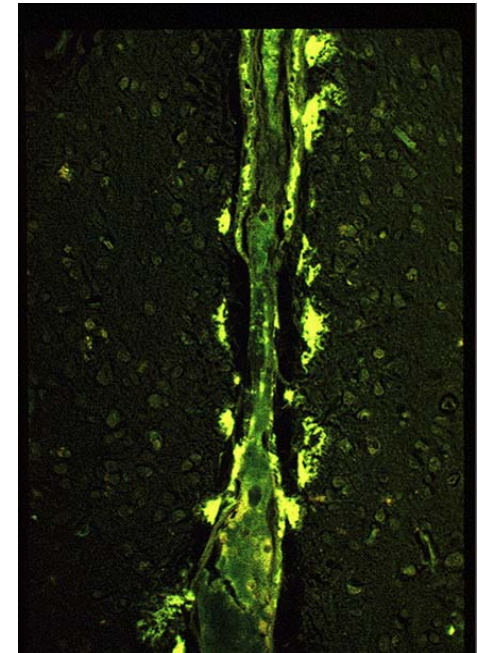


<http://www.adrc.wustl.edu/adrc2.html>



Cerebral Amyloid Angiopathy

- **Cellular degeneration**
 - *Smooth muscle cells*
 - *Endothelial cells*
- **Increased vascular permeability**
 - *Compromised BBB integrity*
- **Inflammatory responses**
- **Increase in vessel-associated monocyte and macrophage lineage cells**
- **Could contribute to vascular damage and elevated immune response**

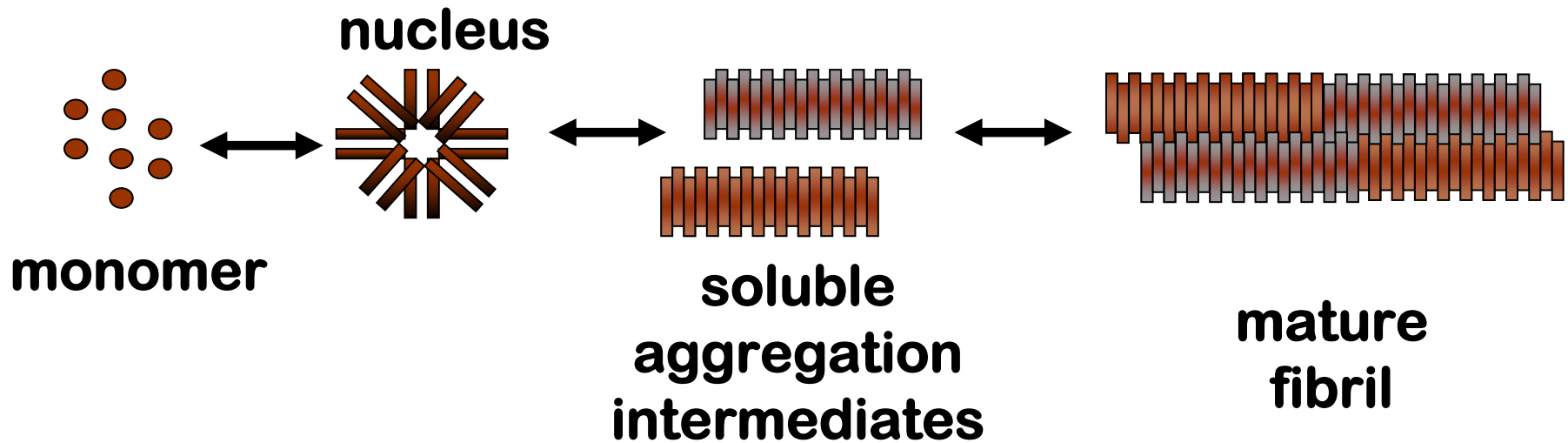


Washington University ADRC

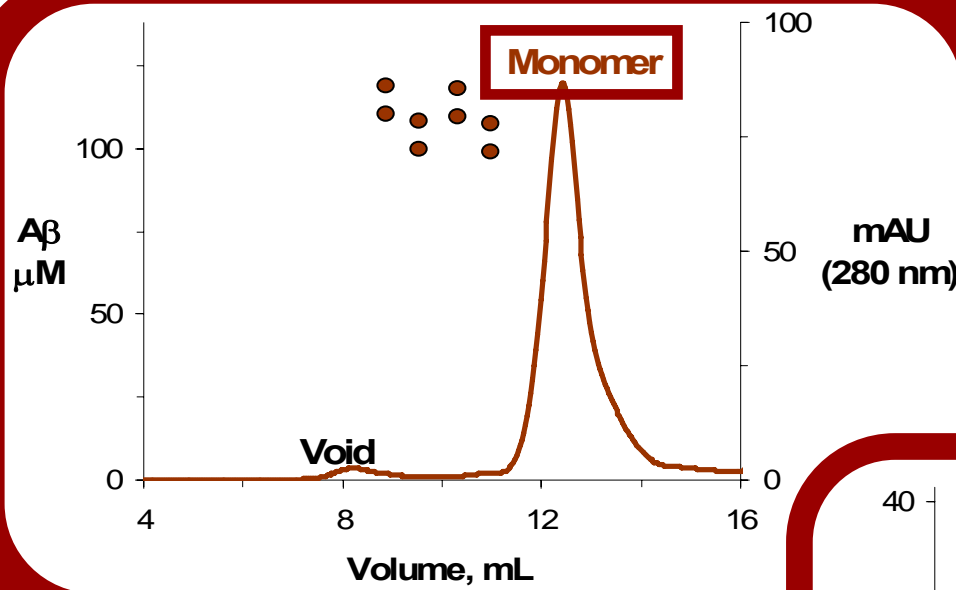


Key Research Question...

What form of A β interacts with endothelial cells to promote activation



Isolation of A β Species

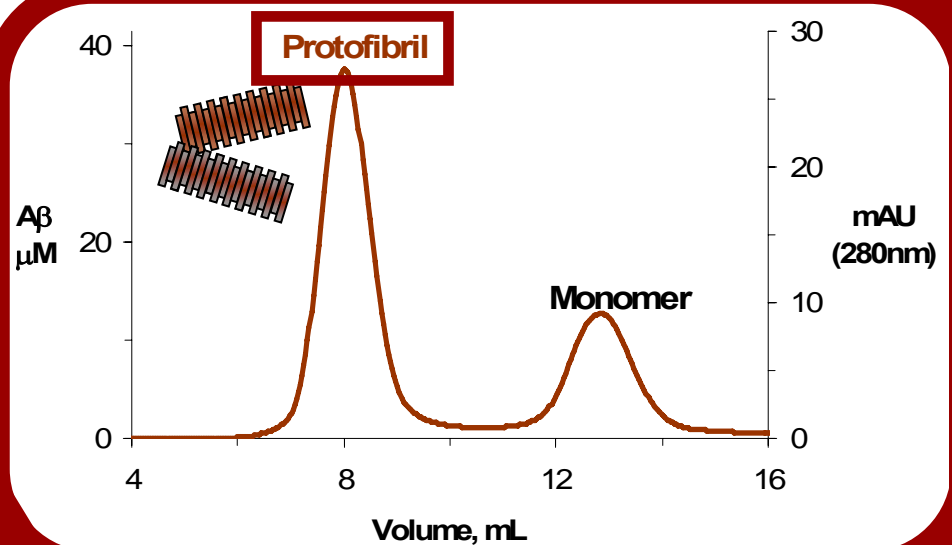


2-10 mM NaCl

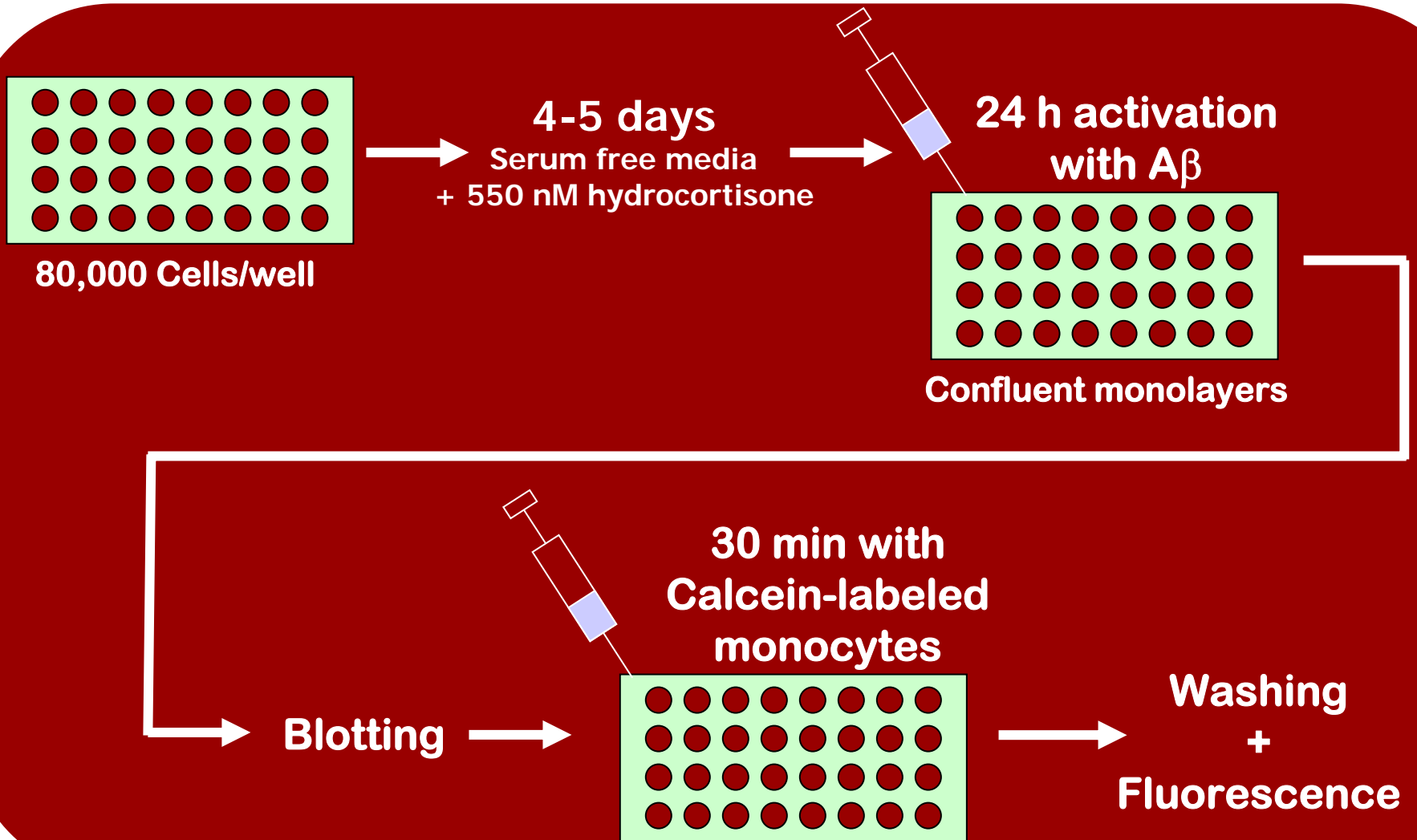
Mixture

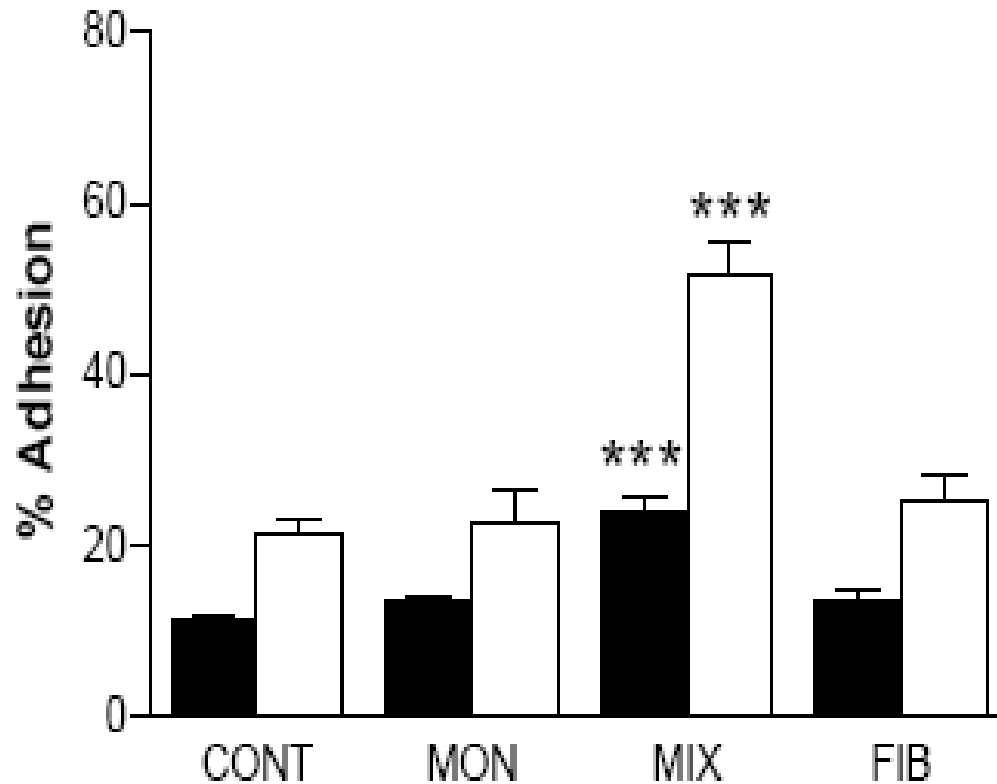
150 mM NaCl

Fibrils



Adhesion Assay



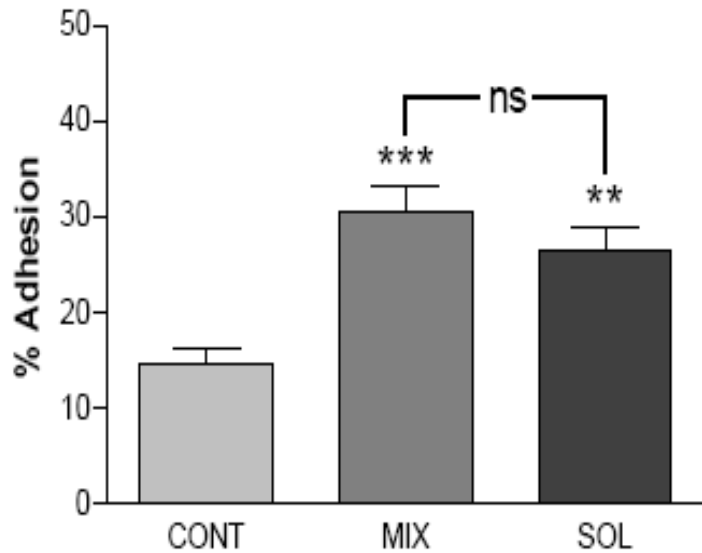


Gonzalez-Velasquez and Moss (2008) *J Neurochem*, 104: 500-513.

Treatment of HBMVEC monolayers with an A β mixture increases monocyte adhesion...

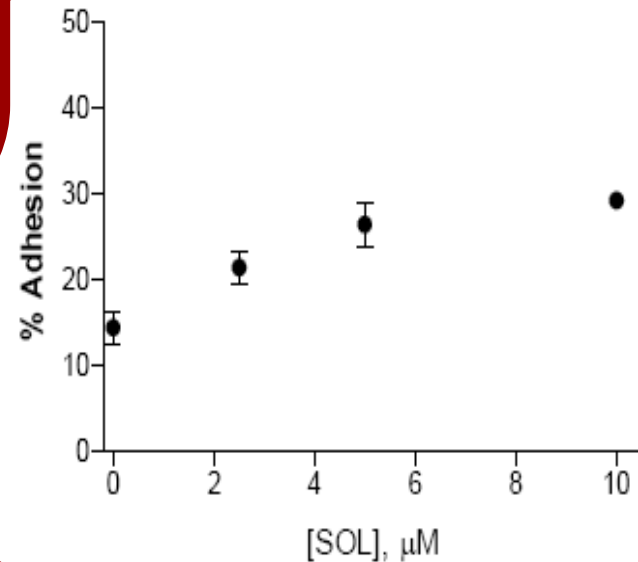
...while isolated monomer and fibril have little effect.





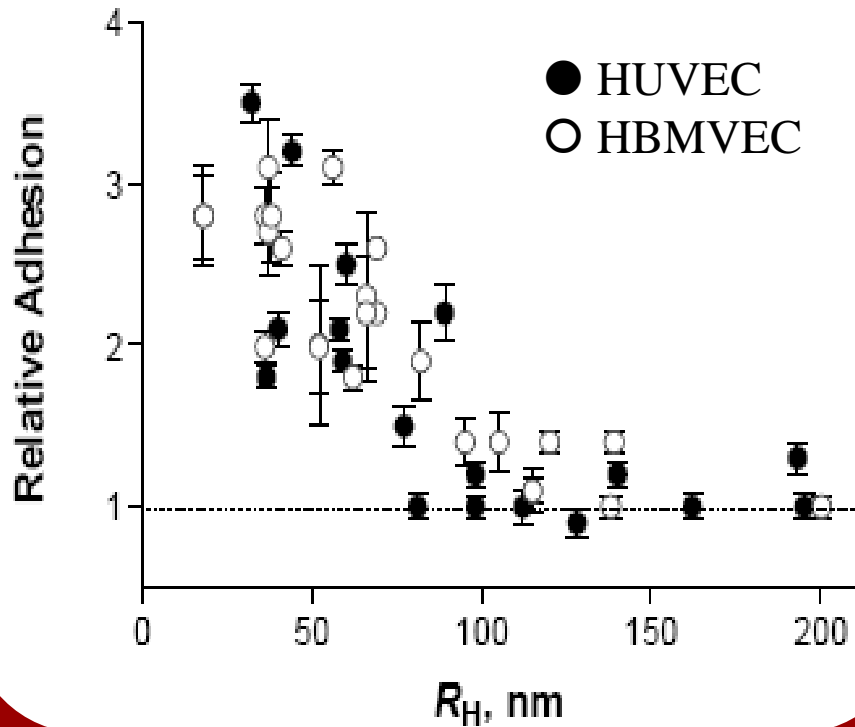
Treatment of HBMVEC monolayers with isolated soluble aggregation Intermediates increases monocyte adhesion...

...in a dose dependent manner.



Gonzalez-Velasquez and Moss (2008) *J Neurochem*, **104**: 500-513.





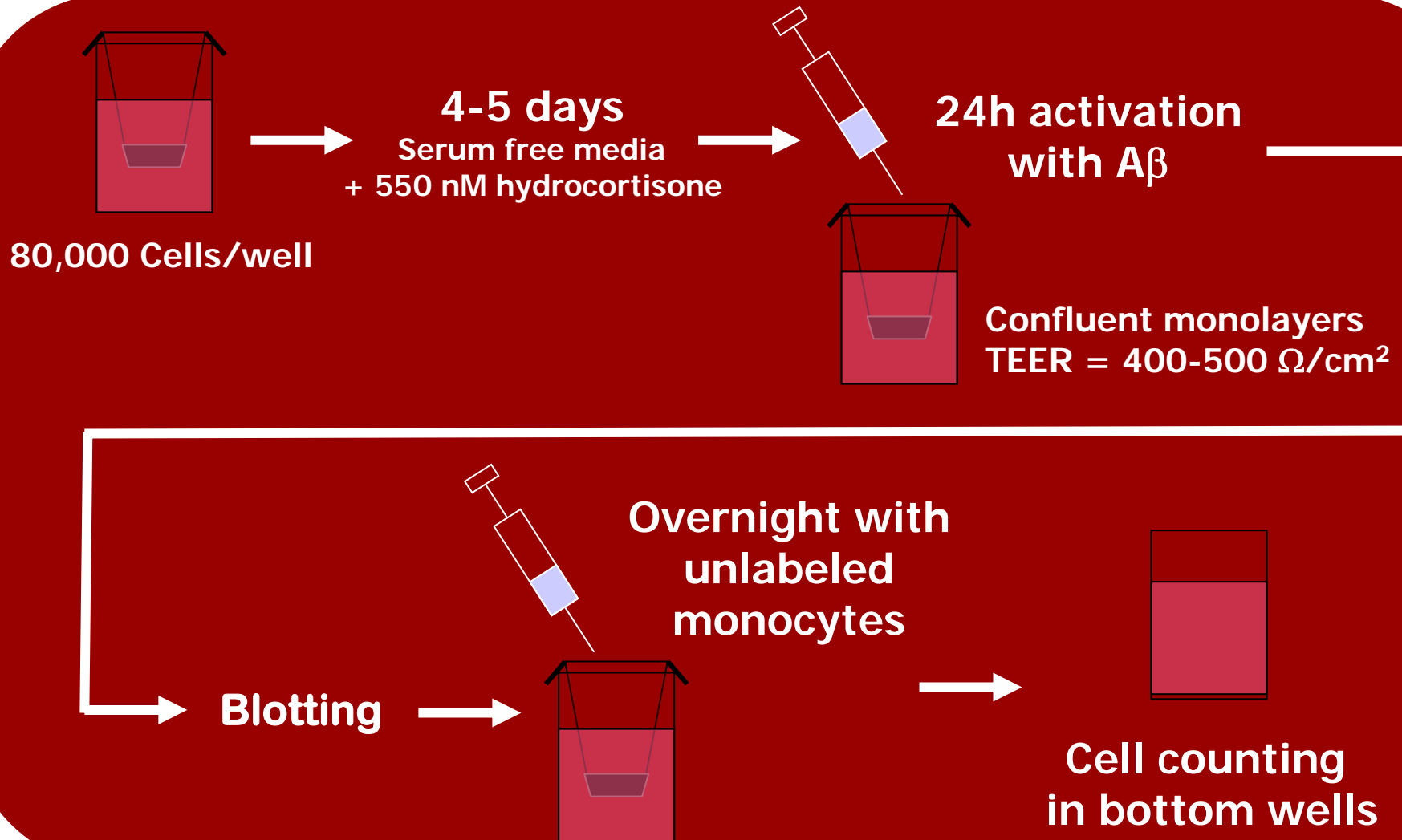
Stimulation of adhesion is more pronounced in the presence of smaller soluble $A\beta$ aggregation intermediates.

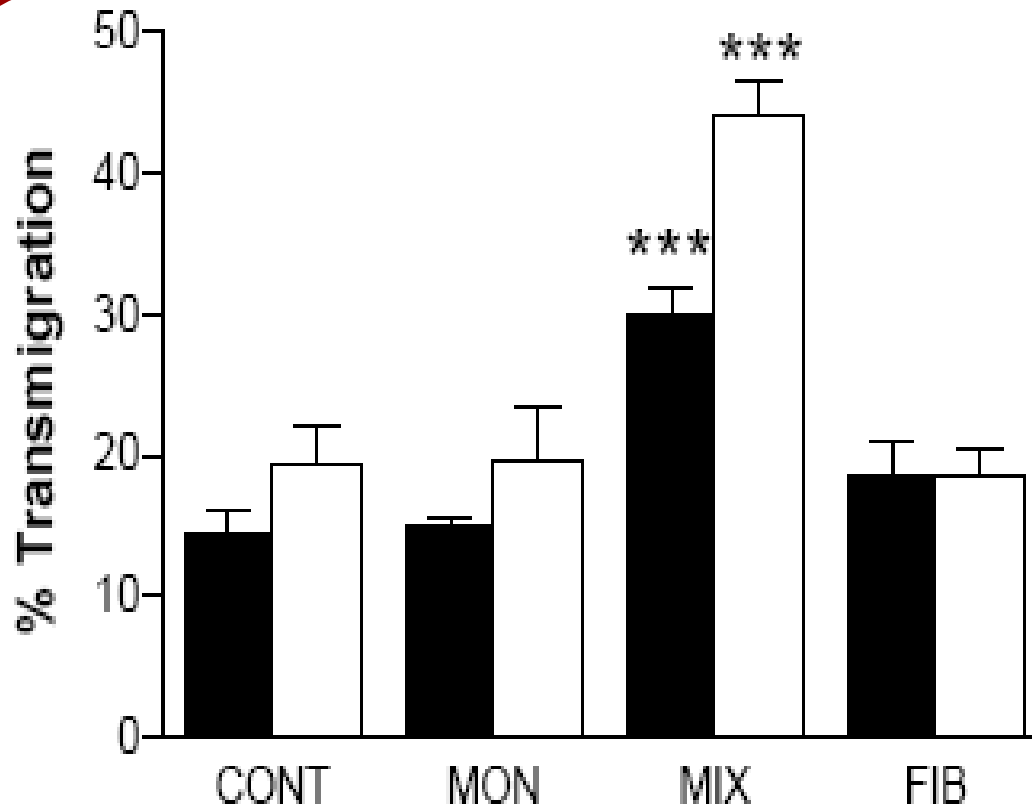
Gonzalez-Velasquez and Moss (2008) *J Neurochem*, **104**: 500-513.



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Transmigration Assay



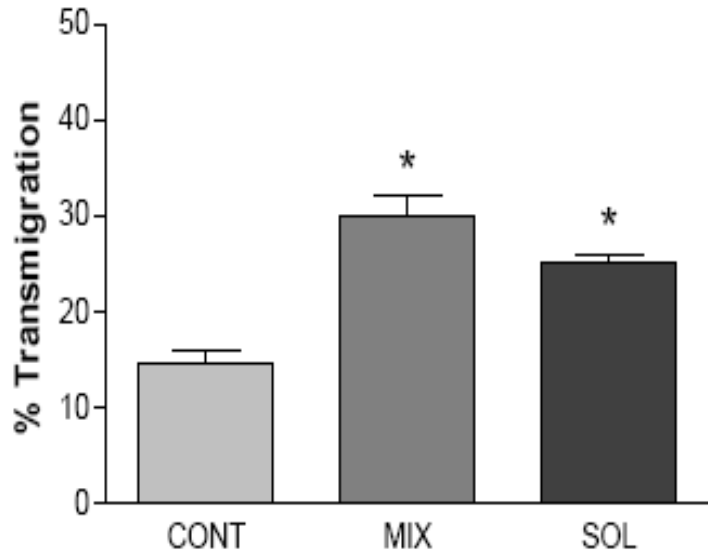


Gonzalez-Velasquez and Moss (2008) *J Neurochem*, 104: 500-513.

Treatment of HBMVEC monolayers with an A β mixture increases monocyte transmigration...

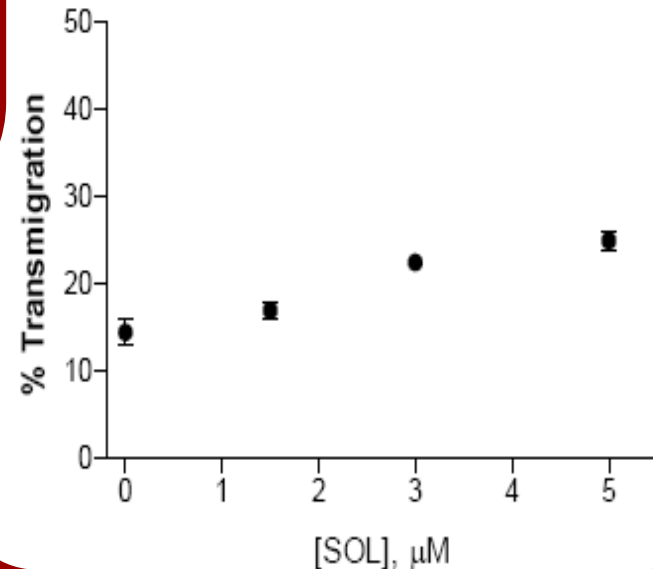
...while isolated monomer and fibril have little effect.





Treatment of HBMVEC monolayers with isolated soluble aggregation Intermediates increases monocyte transmigration...

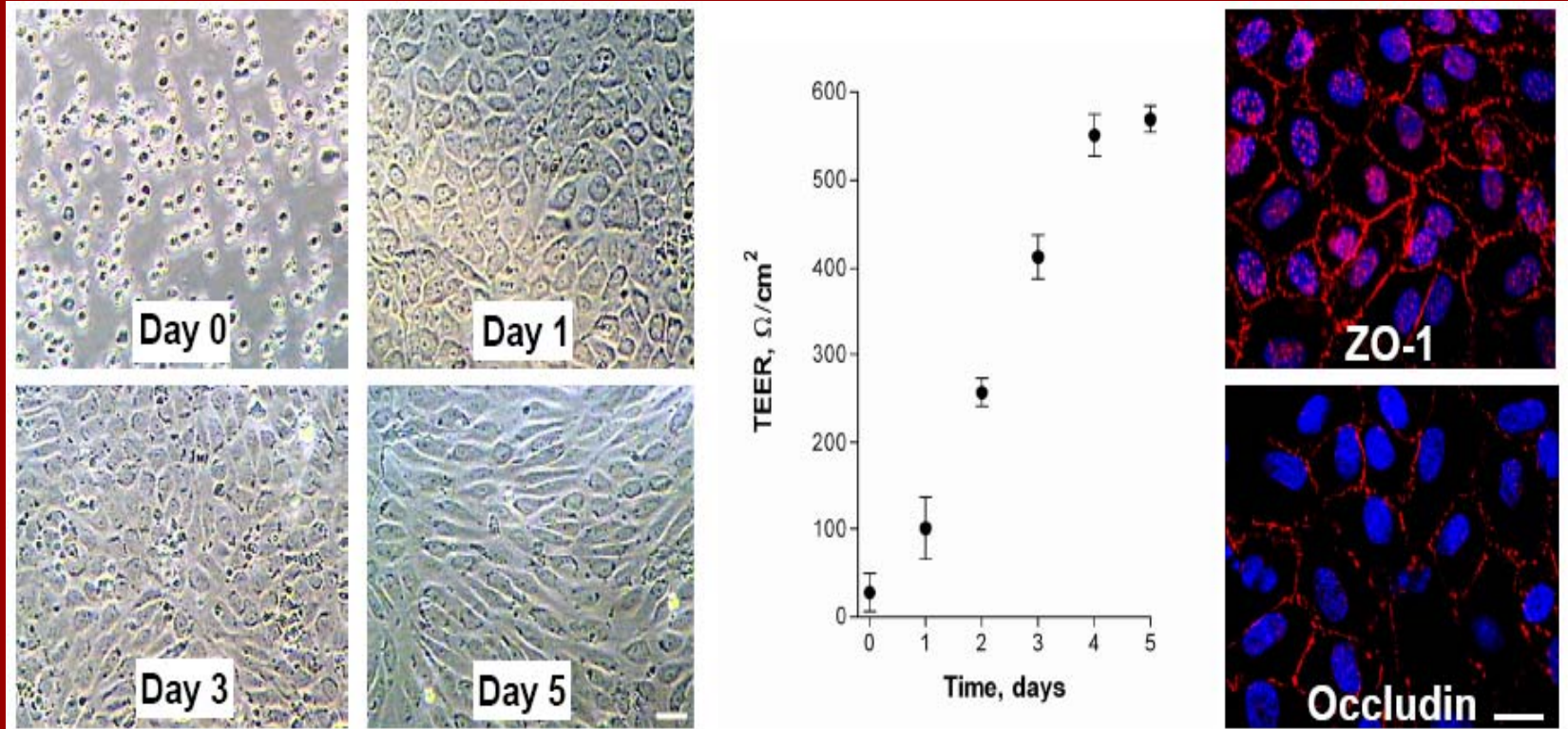
...in a dose dependent manner.



Gonzalez-Velasquez and Moss (2008) *J Neurochem*, **104**: 500-513.



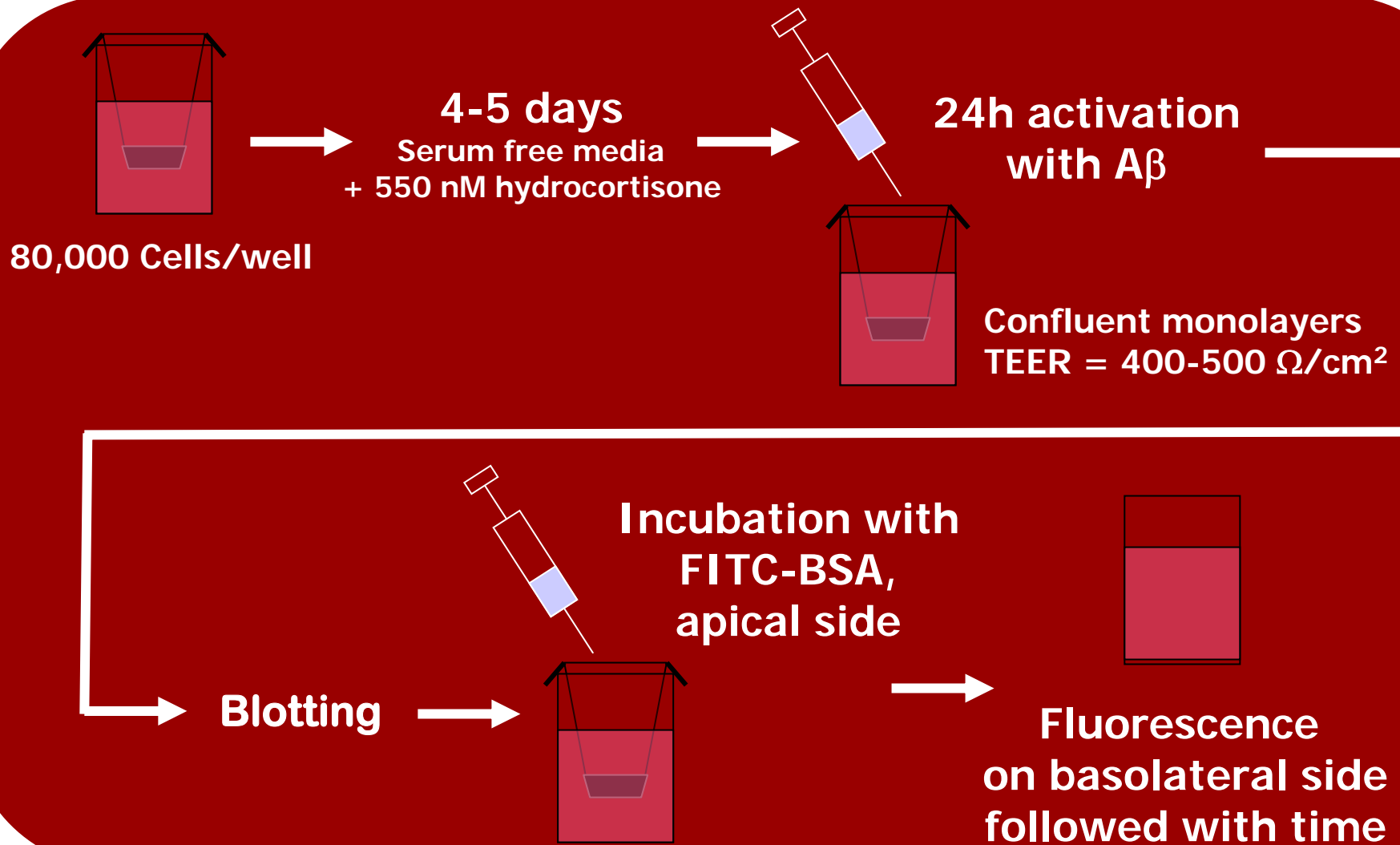
Model endothelium exhibits blood-brain-barrier properties.



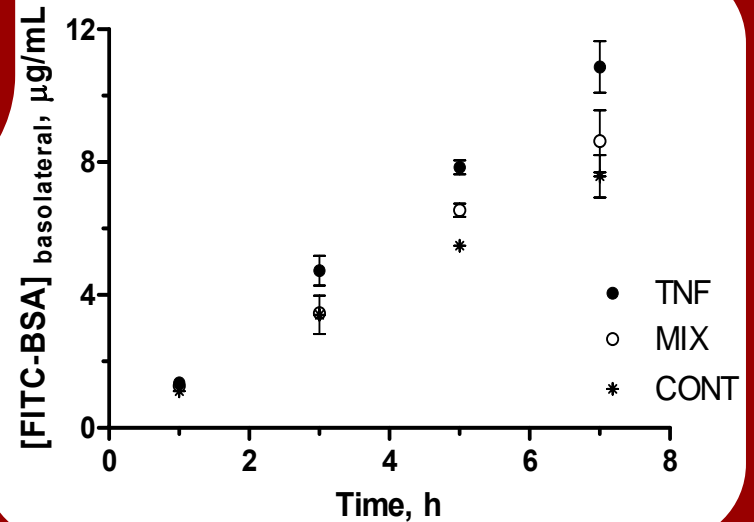
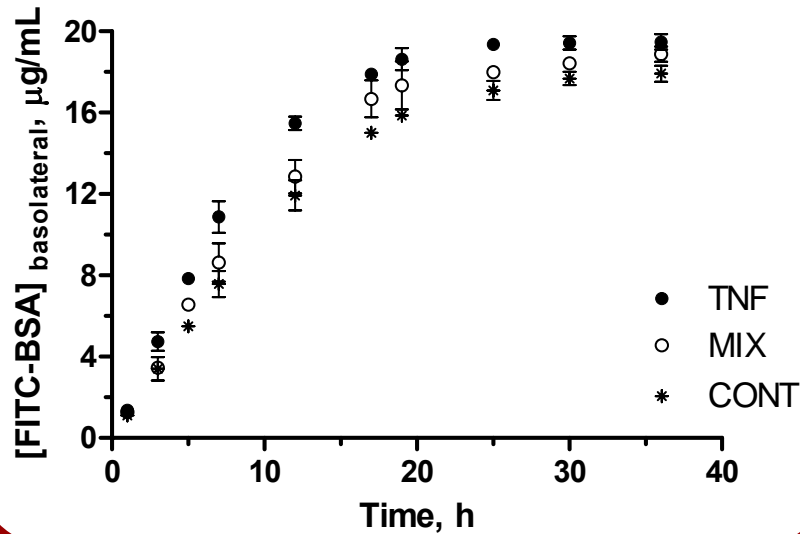
Gonzalez-Velasquez and Moss (2008) *J Neurochem* 104: 500-513.



Permeability Assay



Treatment of HBMVEC monolayers with an A β reaction mixture increases monolayer permeability.



Calculation of Permeability Coefficients

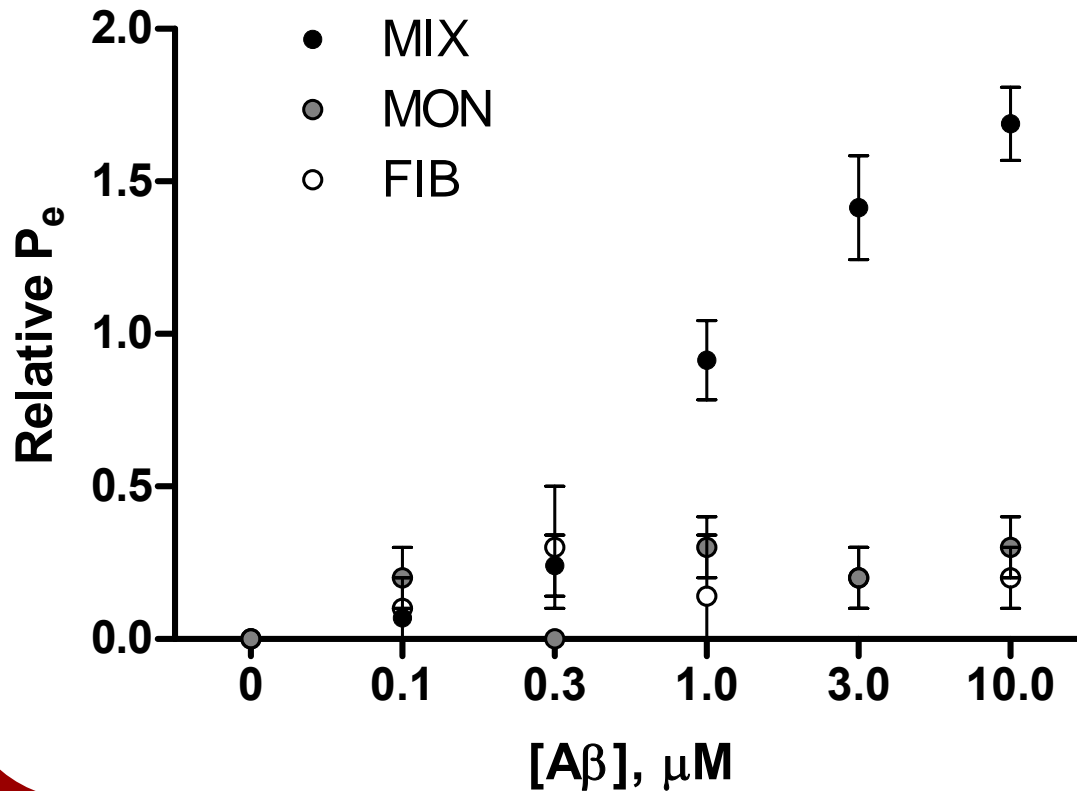
Permeability was determined using a linear approximation of Fick's law:

$$PS = \frac{J}{[BSA]_{apical} - [BSA]_{basolateral}}$$

Permeability coefficients (P_e) are determined following correction for the membrane:

$$\frac{1}{P_e S} = \frac{1}{P_{monolayer} S} - \frac{1}{P_{insert} S}$$



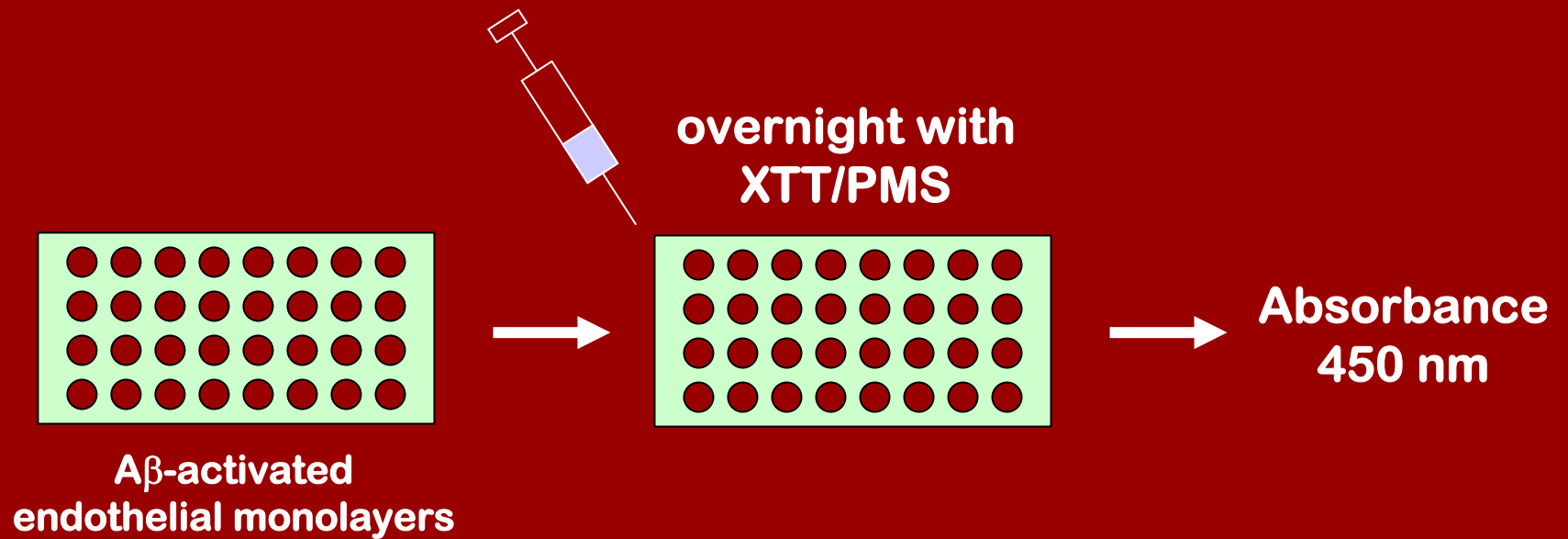


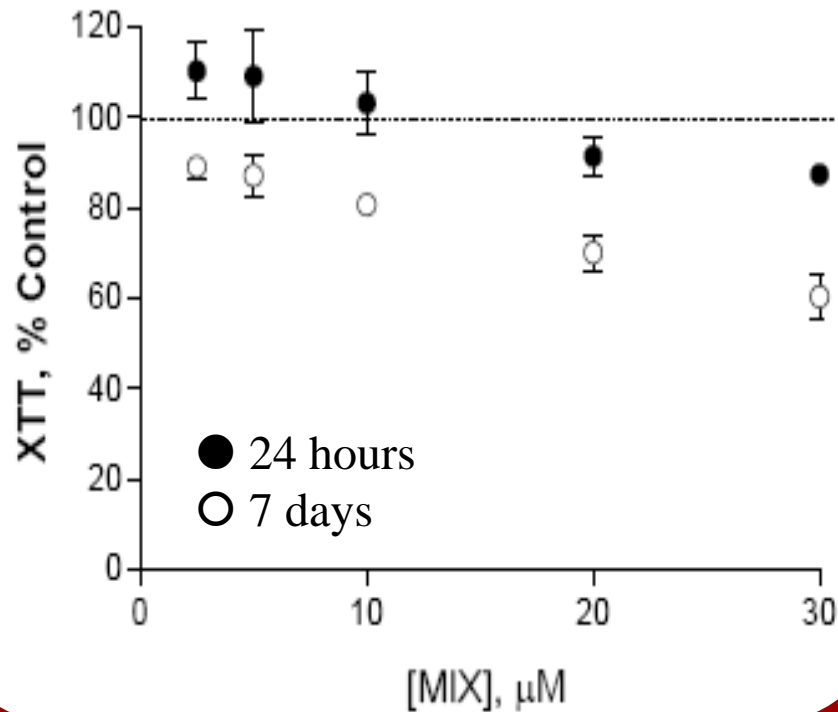
Treatment of HBMVEC monolayers with an $A\beta$ mixture increases monolayer permeability...

...while isolated monomer and fibril have little effect.



Toxicity Assay





Treatment of HUVEC monolayers with an A β mixture fails to induce toxicity at relevant treatment times and concentrations.

Extended treatment times or higher concentrations are required to observe cellular degeneration.

Gonzalez-Velasquez and Moss (2008) *J Neurochem*, **104**: 500-513.



Future Work



Correlate short-term physiological responses of $A\beta$ in endothelial monolayers with long-term neurotoxicity

Exploit these effects in an endothelial cell-based biosensor

Together, these goals will result in development of an endothelial cell-based biosensor for the early detection of AD.



Summary

- **A β is capable of activating endothelial monolayers for adhesion and transmigration.**
 - **Isolated soluble A β aggregates are selectively responsible for activation.**
 - **A β activation correlates inversely with aggregate size.**
- **A β is capable of inducing permeability in endothelial monolayers**
 - **Soluble A β aggregates are likely responsible for increases in permeability.**
- **The selective effects of soluble A β species in endothelial monolayers may be exploited for use in a cell-based biosensor.**



Acknowledgements

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Chen Suo

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Fahmin Basher

Tim Davis

Sarah Holton

Christie Long

Brandon Murphy



**Selective activation of endothelial monolayers
by soluble aggregates of the amyloid- β protein:
Potential for exploitation in a cell-based sensor**

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