Gold Nanoparticles for Ultrasensitive and Selective Detection of Alzheimer’s Tau Protein and Acetylcholinesterase in Cerebrospinal Fluid

Presenter : Septila Renata
Coach Professor : Dr. Yun-Ru (Ruby) Chen
Sit-in Professor : Dr. Su-Chang (Tom) Lin
Coordinator : Dr. Hsien-Ming Lee
A Highly Sensitive Gold-Nanoparticle-Based Assay for Acetylcholinesterase in Cerebrospinal Fluid of Transgenic Mice with Alzheimer’s Disease

Dingbin Liu, Wenwen Chen, Yue Tian, Sha He, Wenfu Zheng, Jiashu Sun, Zhuo Wang,* and Xingyu Jiang*

A SERS-Based Sandwich Assay for Ultrasensitive and Selective Detection of Alzheimer’s Tau Protein

Adem Zengin,† Uğur Tamer,‡ and Tuncer Caykara*†

†Department of Chemistry, Faculty of Science, Gazi University, 06500 Besevler, Ankara, Turkey
‡Department of Analytical Chemistry, Faculty of Pharmacy, Gazi University, 06330 Etiler, Ankara, Turkey
What is Alzheimer’s Disease?

- AD is the most common cause of dementia among people age 65 and older.
- For every 5-year age group beyond 65, the percentage of people with AD doubles.
- By 2050, 13.2 million older Americans are expected to have AD if the current numbers hold and no preventive treatments become available.
Adding up to Alzheimer’s Disease.
The known genetic causes of Alzheimer’s disease (left side) are responsible for only a small number of the total cases. The possible environmental factors (right side) are largely speculative; none are proven causes. The most likely pathway resulting in Alzheimer’s disease in the general population (bottom) involves predisposing genetic factors (such as the ε4 variant of the APOE gene) combined with a variety of environmental factors and interacting with physiological aging processes in the brain.
The Brain and Alzheimer’s Disease

Two major structural changes:

1. **Neurofibrillary tangles**  
   - Bundles of twisted threads that are the product of collapsed neural structures (contain abnormal forms of *tau* protein)

2. **Amyloid plaques**  
   - Dense deposits of deteriorated *amyloid* protein, surrounded by clumps of dead nerve and glial cells
tangle
plaques
Alzheimer cells
healthy cells
Alzheimer disease – molecular pathogenesis
Alzheimer disease – molecular pathogenesis
What Happen to the Brain?

- Progressive loss of cognitive function is accompanied by pathologic (disease-associated) changes in the brain.
- Formation of plaques (tiny brillo pads) in the space between the nerve cells. Comprised of beta-amyloid.
- The protein, tau, which normally channels chemical messages inside nerve cells, deforms and collapses into neurofibrillary tangles like twisted bits of thread inside nerve cells.
- As the disease progresses, nerve cells in several brain areas shrink and die, including cells that normally produce critical neurotransmitters. These chemical messengers relay brain signals from one nerve cell (neuron) to another.
- Acetycholine (neurotransmitter) is deficient in people with Alzheimers.
- As nerve cells continue to die, the brain shrinks itself. In some cases an Alzheimer’s brain can actually be 1/3 the size of a non-afflicted brain!
CEREBROSPINAL FLUID (CSF)

- CSF found in cerebral ventricles and cisterns and in subarachnoid space surrounding brain and spinal cord

- Major functions of CSF:
  - Provides support
  - Regulates ionic composition
  - Removes metabolites

- Alterations in CSF composition present in various disorders

- In adults, total volume of CSF in all spaces combined is normally about 150 mL

- Between 400–500 mL of CSF is produced and reabsorbed daily
What is Cerebrospinal Fluid (CSF)?

- 70% CSF produced in choroid plexuses of lateral, third and fourth ventricles
- Produced at rate of 500 cc/day or approximately 20cc/hour (0.3-0.5 cc/kg/hr)
- Eliminated by being absorbed into the arachnoid villi --> dural sinus --> jugular system
- Other sources of CSF production from capillary ultrafiltrate (Virchow-Robin spaces)
- Additionally some produced from metabolic H₂O production
Cerebrospinal Biomarker

- Cerebrospinal Fluid can be used to direct target for AD biomarker
  - Its straight contact with extracellular space of the brain where many biochemical processes in the brain take place and are reflected in the CSF
  - Low level of Acetylcholinesterase in CSF indicate in preclinical stage of Alzheimer Disease

- Cerebrospinal Fluid biomarkers that used to aid the diagnosis of Alzheimer’s disease:
  - increase in phosphorylated tau (P-tau$_{181}$)
  - increase in total tau (T-tau)
  - decrease in Aβ$_{1-42}$
What is Acetylcholinesterase (AChE)

- An enzyme with a sulfhydryl active site
- Hydrolyses acetylcholine at cholinergic synapses
- Present in the autonomic, central and peripheral nervous systems
Structure of AChE

The active site of AChE is made up of **two subsites**, both of which are critical to the breakdown of ACh. The **anionic site** serves to bind a molecule of ACh to the enzyme. Once the ACh is bound, the hydrolytic reaction occurs at a second region of the active site called the **esteratic subsite**. Here, the ester bond of ACh is broken, releasing acetate and choline. Choline is then immediately taken up again by the high affinity choline uptake system on the presynaptic membrane.
Method to Measure Level of AChE

- Chemiluminescent of Fluorescent Assay
  - Lack sufficient sensitivity
  - Require tedious chemistry synthesis
- Electrochemical probes
- AuNPs-based Colorimetric Assay
  - Difficult to control the aggregation of AuNPs
- RB-AuNPs-based Colorimetric and Fluorometric Assay
  - Improve sensitivity and accuracy of assay
RB is an ideal ligand, because water soluble, photostable, strongly fluorescent, and readily adsorb onto surface of AuNPs
Characterization

Zeta potential before (red) and after (green) adding AChE

RB-AuNPs is negative because of acidic group on RB. After added AChE, zeta potential increased because of presence of positive charge ammonium group in the thiocholines derived from AChE catalyzed hydrolysis of ATC.
Sensitivity of Assay for AChE

Detection limit can reach 1 mU/mL

Change in UV-VIS quantified by ratio A/D

Degree of aggregation of RB-AuNPs is proportional to the concentrations of AChE and the incubation time.
Sensitivity of Assay for AChE

Change in fluorescence spectra quantified by ratio $F/F_0$

Detection limit can reach 0.1 mU/mL
Selectivity of Assay for AChE

Detection for other biothiols and proteins that particularly exist in human body

It show the advantage of dual readout in this assay, from Fluorometric and Colorimetric
Application in CSF of Transgenic Mice

- Transgenic senescence-accelerated mouse prone/8 (SAM-P8) as model for age-related dementia of the Alzheimer phenotype
- Transgenic senescence-accelerated mouse resistant/1 (SAM-R1) as model of normal aging characteristic

Treated with neostigmine (Alzheimer drug)

Pretreated with galantamine (AChE inhibitor)

If AChE inhibitor present, AChE fail to catalyze ATC to generate thiocholine that is required to cause aggregation of RB-AuNPs, so the color of solution remain red
Application in CSF of Transgenic Mice

UV-VIS study

Fluorescence study
Calibration Curve

Smallest volume of SAM-P8 CSF sample where AChE level in undetectable is 0.125 µL as background.

AChE level in 4 CSF sample can be calculated:
- Sample 1 = 3.1 U/mL
- Sample 2 = 0.56 U/mL
- Sample 3 = 1.1 U/mL
- Sample 4 = 0.85 U/mL
CONCLUSION

- RB-AuNPs with dual readout (colorimetric and fluorometric) can present a highly sensitive and selective assay for Acethylcholisterase detection in CSF.

- Monitoring acethylcholinesterase in human CSF could be useful for early diagnostic of AD, especially combined with neuroimaging technique or CSF biomarker detection.
A Highly Sensitive Gold-Nanoparticle-Based Assay for Acetylcholinesterase in Cerebrospinal Fluid of Transgenic Mice with Alzheimer’s Disease

Dingbin Liu, Wenwen Chen, Yue Tian, Sha He, Wenfu Zheng, Jiashu Sun, Zhuo Wang,* and Xingyu Jiang*

A SERS-Based Sandwich Assay for Ultrasensitive and Selective Detection of Alzheimer’s Tau Protein

Adem Zengin,† Uğur Tamer,‡ and Tuncer Caykara*,†

†Department of Chemistry, Faculty of Science, Gazi University, 06500 Besevler, Ankara, Turkey
‡Department of Analytical Chemistry, Faculty of Pharmacy, Gazi University, 06330 Etiler, Ankara, Turkey
Cerebrospinal Biomarker

Cerebrospinal Fluid biomarkers that used to aid the diagnosis of Alzheimer’s disease:

- Increase in phosphorylated tau (P-tau$_{181}$)
- Increase in total tau (T-tau)
- Decrease in Aβ$_{1-42}$
Tau Protein

- A microtubule associated protein largely localized to neuronal axons
- Important biological role in stabilizing microtubules and thereby aiding neuronal structure and axonal transport
- Hyperphosphorylation of tau at specific serine & threonine epitopes impairs its normal function; this process either leads to neuronal dysfunction & death, or is a marker of neuronal death
**Tau protein: Production of Tangles**

- Tau proteins promote microtubule stability
- Abnormal tau protein binding causes microtubule breakdown and the tau protein clump to form neurofibrillary tangles
- Tau proteins become hyperphosphorylated
Detection Of Tau Protein

- Enzyme-Linked Immunosorbent Assay (ELISA)
- Localized Surface Plasmon Resonance (LSPR) technique
- Chromatography

**DRAWBACK**: require a complicated sample preparation or multiple steps
- Time Consuming
- Costly

- SERS-Based Sandwich Assay
  - monoclonal antitau functionalized hybrid magnetic nanoparticles as recognition of Tau protein
  - Polyclonal antitau immobilized Gold NPs as SERS component
Surface-enhanced Raman Scattering (SERS)

**SERS Advantage:**
- **Molecular fingerprinting**
  Unique vibrational spectra distinguishes molecules
- **Multiplexed sensing**
  Plasmon resonances allow for sensor tenability
- **In vivo applicability**
  Near-IR excitation and biocompatibility allow
- **Femtomolar and beyond**
  Single molecule spectroscopy is possible
- **Sensitive and surface selective**

**SERS Enhancement:**
- **Electromagnetic enhancement**
  Gold NP
- **Chemical Enhancement**
  DTNB (5,5-dithiobis (2-dinitrobenzoic acid) as Raman tag

- **Advantage:**
  - Low detection concentrations
  - Rapid measurement
  - High sensitivity for targeting Tau Protein
Scheme of the Synthesis of Hybrid Nanoparticles

Figure 1. TEM images of (a) oleic acid-stabilized MNPs, (b) silica-coated MNPs, and (c) hybrid MNPs (polymerization time is 72 h).
Preparation of Sandwich Assay

Tem image of the sandwich complex
In concentration 25 fM, characteristic peak at 1332 cm\(^{-1}\) become difficult to distinguish from that in blank spectrum. Detection limit is 25 fM.
Selectivity for Tau-protein

SERS intensity obtained from complex and Tau had no apparent different, indicating that BSA and IgG had almost negligible on the Tau protein
FUTURE WORK

- Apply SERS-based sandwich assay for tau detection in vivo
- Apply SERS-based sandwich assay to detect other CSF biomarker
Thank you.