SRIP Team 29: See Jiat Gabriel, Lee Jie Rong Jerome, Yung Jia Hui
LKCMedicine, MBBS Programme, Year 3
Supervisor: Prof Balázs Gulyás
Advisor: Dr Parasuraman Padmanabhan

Bifunctional Fluorescent/Raman Nanoprobe for the Early Detection of Amyloid

Yang Xia, Parasuraman Padmanabhan, Sreelatha Sarangapani, Balázs Gulyás & Murukeshan Vadakke Matham

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1. INTRODUCTION

Neurodegenerative disorders are caused by neuronal death, which can damage patient's memory and other cognitive capabilities. The most common neurodegenerative disorder is Alzheimer's disease (AD)[1].

AD is most widely accepted to be caused by the Amyloid hypothesis. Amyloid plaques are insoluble, extracellular aggregates formed by misfolded peptides[2] which aggregate to produce toxic molecules, leading to neuronal death.

Brain amyloid is the most definitive histopathologic hallmark for AD, formed by amyloid beta (Aβ) peptides. Aβ peptides have various lengths, but the major contributor to AD is Aβ42.[3]

As amyloid aggregation may start long before the appearance of detectable clinical changes, this study aims to evaluate the use of fluorescence imaging and Raman nanoprobes in the early detection of amyloid peptides. This can be expected to be utilized for the early diagnosis of AD.[4]

2. MATERIALS AND METHODS

1) Preparation of RB-AuNP complex Rose Bengal (RB) was conjugated to amine-functionalized gold nanoparticles (AuNPs) through a two-step reaction.

2) Cytotoxicity well assay HEK-293 cells were seeded into a 96-well plate with DMEM/F-12 medium and refreshed with PBS or various concentrations of RB-AuNP. Comparison was made against reference Thioflavin T Fluorescence (3a) and investigation of the viability of seRs (3b).

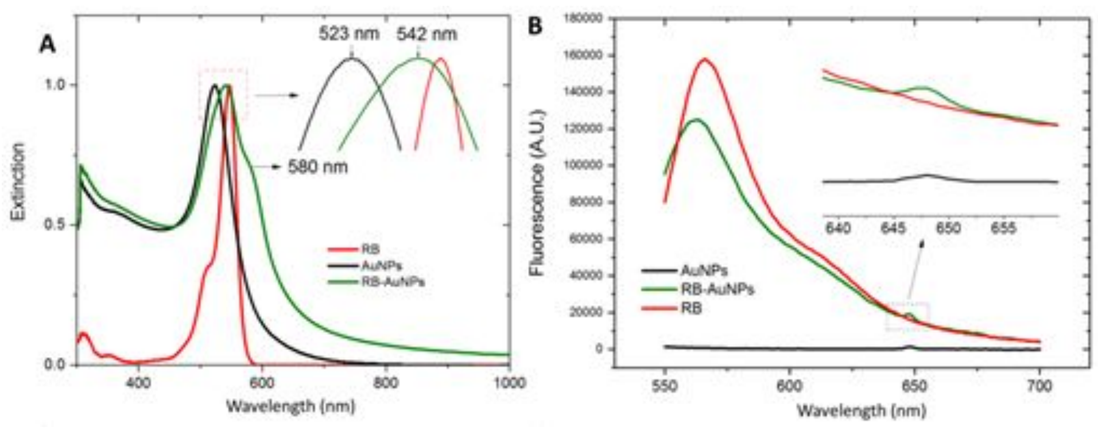
3a) Fluorescent detection of Aβ42 peptides & RB-AuNPs Different concentrations of Aβ42 peptides were mixed with 1 μg/ ml of RB-AuNPs, and fluorescence emission was recorded. Mouse brain slices were stained with RB-AuNPs or the reference ThT solution. Pearson's linear Correlation Coefficient (PCC) and Manders coincidence were calculated.

3b) seRs Detection of Aβ42 peptides Concentrations of Aβ42 peptides was mixed with 1 μg/ml of RB-AuNPs and left to dry for 6 hours, and Raman spectra of dried samples were measured.

3. RESULTS AND DISCUSSION

Characterisation of RB-AuNPs

After the absorption process, there was a shift in the apparent Zeta potential from −8.43±3.68 mv to −17.2±7.32 mv, and a change in the emission spectrum, indicating absorption of RB to the AuNPs.

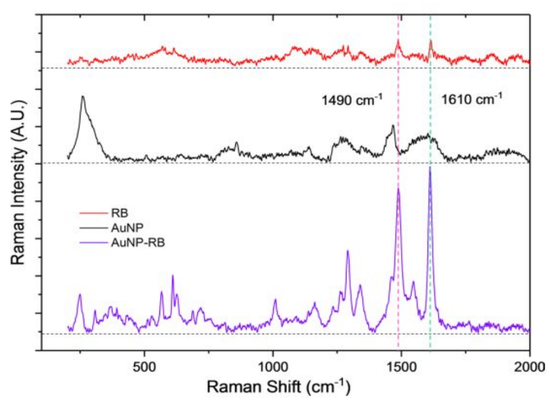


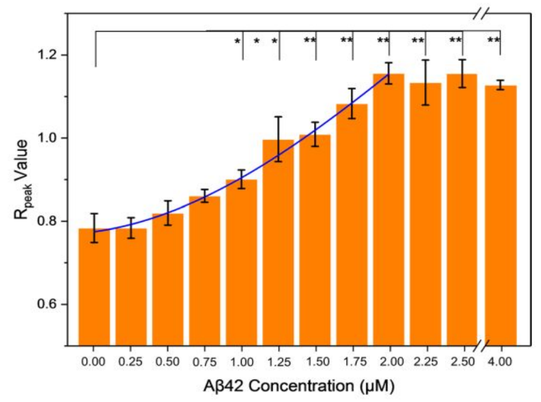
3. RESULTS AND DISCUSSION

Raman spectrum interaction with Aβ42 peptides

The Raman spectrum of RB-AuNPs has a significant SERS effect with 2 major peaks located at 1490 and 1610 cm^{-1}. The ratio between the intensity at 1490 cm^{-1} and 1610 cm^{-1} (R_{peak}) is 0.780 in non-exposed RB-AuNPs.

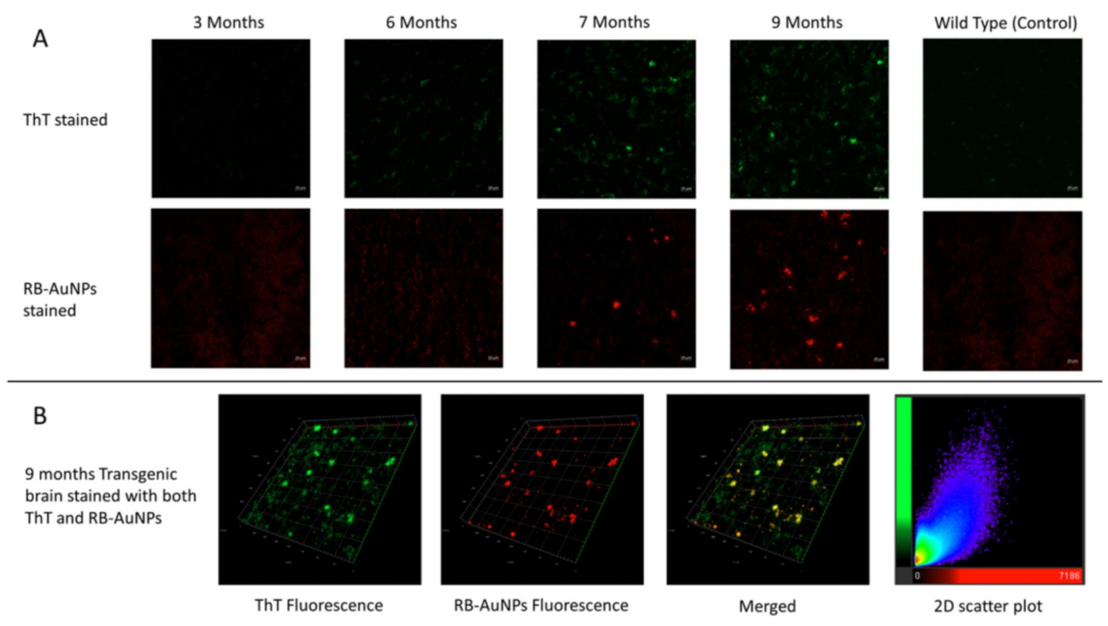
When mixed with Aβ42 peptides, there is a change in the R_{peak} value, with a clear positive correlation with Aβ42 concentration up to 2μM (r^2=0.9739), demonstrating that the Raman spectrum of RB-AuNPs can be used to identify Aβ42 peptides.





Fluorescence imaging

The fluorescence emission of RB-AuNPs increases with Aβ42 concentration. Comparison of RB-AuNPs with ThT was performed using fluorescence imaging in 3xTg mice of different ages. 93.62% of the RB-AuNPs fluorescence coincided with the ThT fluorescence, showing that RB-AuNPs are sufficient for the labelling of amyloid plaques in mouse brain slices.



Biocompatibility assay

The RB-AuNPs when incubated with HEK 293 cells at a high incubation concentration of 25μg/ml had a cell viability greater than 95% with intact cell morphology, indicating biocompatibility.

4. CONCLUSION

This study demonstrates the feasibility of RB-AuNPs in the early diagnosis of Aβ42 peptides in ex vivo specimens.

Possible future research can be expanded towards the assessment of RB-AuNPs with in vivo studies and the exploration of different methods of functionalizing RB-AuNPs to be visualised with other techniques such as radionuclide imaging.

5. REFERENCES

1. Prince, M., Comas-Herrera, A., Knapp, M., Guerchet, M. & Karagiannidou, M. World Alzheimer report 2016: improving healthcare for people living with dementia: coverage, quality and costs now and in the future (2016).
2. Rambaran, R. N. & Serpell, L. C. Amyloid fibrils: Abnormal protein assembly. *Prion* **2**, 112–117 (2008).
3. Luheshi, L. M. *et al*. Systematic *in vivo* analysis of the intrinsic determinants of amyloid β pathogenicity. *PLoS biology* **5**, e290 (2007).
4. Kung, H. F. The β-amyloid hypothesis in Alzheimer's disease: seeing is believing. *ACS medicinal chemistry letters* **3**, 265–267 (2012).

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