ARL-TN-0753 ● APR 2016

US Army Research Laboratory

In-vitro Synthesis of Gold Nanoclusters in Neurons

by Maggie Gillan and Nicole Zander

NOTICES

Disclaimers

The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorized documents.

Citation of manufacturer's or trade names does not constitute an official endorsement or approval of the use thereof.

Destroy this report when it is no longer needed. Do not return it to the originator.

ARL-TN-0753 ● APR 2016

US Army Research Laboratory

In-vitro Synthesis of Gold Nanoclusters in Neurons

by and Nicole Zander
Weapons and Materials Research Directorate, ARL

Maggie Gillan
Oak Ridge Institute for Science and Education, Oak Ridge, TN

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED (From - To)
April 2016	Technical Note	1 August 2013–31 October 2014

4. TITLE AND SUBTITLE
In-vitro Synthesis of Gold Nanoclusters in Neurons

5a. CONTRACT NUMBER

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Maggie Gillan and Nicole Zander

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
US Army Research Laboratory
ATTN: RDRL-WMM-G
Aberdeen Proving Ground, MD 21005-5069

8. PERFORMING ORGANIZATION REPORT NUMBER
ARL-TN-0753

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution is unlimited.

13. SUPPLEMENTARY NOTES

14. ABSTRACT
Gold nanoclusters (AuNCs) synthesized intracellularly have potential for use as in-vivo or in-vitro pressure probes for evaluating the effects of traumatic brain injury. AuNCs were grown within NG-108-15 neuroblastoma-glioma hybrid cells, indicated by increased fluorescence intensity at 488 nm. Effect of gold salt concentration on fluorescence intensity was evaluated. Cell viability at 24-h postgold salt addition was tested via an MTS cell viability assay.

15. SUBJECT TERMS
gold nanoclusters, neurons, in-vitro synthesis, fluorescence, viability

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Nicole Zander
Unclassified	Unclassified	Unclassified	UU	14	19b. TELEPHONE NUMBER (Include area code) 410-306-1965

Contents

List of Figures iv

1. Introduction 1

2. Materials and Methods 2

3. Results and Discussion 2

4. Conclusions 5

5. References 6

Distribution List 7

List of Figures

Fig. 1 Fluorescence, phase contrast, and combined confocal micrographs of NG108-15 cells 24 h after addition of a) 0 mM, b) 0.1 mM, c) 1 mM, and d) 10 mM of gold salt 3

Fig. 2 Fluorescence intensity data from NG108-15 cells incubated with gold salt for 24 h 4

Fig. 3 Viability as determined by a MTS assay of NG108-15 cells incubated with varied concentrations of $HAuCl_4$ salt 5

1. Introduction

Noble metal nanoclusters (NCs) composed of a few hundred atoms are an emerging class of luminescent nanomaterials. These NCs typically have diameters less than 2 nm, approaching the Fermi wavelength of electrons, which leads to very unique optical, electrical, and chemical properties compared with nanoparticles.[1–3] In particular, they have strong photoluminescence and tunable emission wavelengths. Conjugation of the NCs with proteins stabilizes the system but also is known to alter the fluorescence intensity under pressure due to ligand conformation changes for select gold NC (AuNC) systems.[4] Thus, these nanomaterials could conceivably be used as nano-pressure sensors in a variety of biological and material applications.

One pertinent application is in the study of traumatic brain injury (TBI), which affects over 1.7 million people in the United States annually.[5] The majority of TBIs are classified as mild or mTBIs and account for 80% of all head injuries.[6] mTBIs are rarely accompanied with neuroanatomical abnormalities detectable by conventional imaging methods such as magnetic resonance imaging.[5] Thus, diagnosis relies heavily on self-reported symptoms and is often undiagnosed even though mTBIs and particularly repetitive mTBIs have a significant effect on cognitive function. Frequently, Soldiers in particular are unaware that they have even suffered a TBI and are often treated for posttraumatic stress disorder, since the symptoms at least initially are the same. But mTBI is actually physical brain damage and should follow a very different treatment path. One of the reasons for this problem is the lack of good pressure sensors on the Soldier to declare when he or she has experienced a probable TBI.

The aforementioned NCs with appropriate ligands could potentially serve as a useful tool to diagnose pressure doses. Protein NCs are typically synthesized by traditional wet chemistry methods but have recently been shown to be synthesized in-vitro by mammalian cell lines.[7] These could potentially be isolated from the cells or used for in-vitro TBI research and living pressure sensor devices. This work details the in-vitro synthesis of AuNCs inside a neuronal and glial co-culture cell line (NG108-15) and the effect of gold salt concentration on fluorescence intensity and viability.

2. Materials and Methods

NG108-15 neuroblastoma-glioma hybrid cells (108CC15) (ATCC HB-12317) were maintained in culture in Dulbecco's Modified Eagle Medium supplemented with 1% sodium hypoxanthine, aminopterin, and thymidine supplement and 10% fetal bovine serum. Cells were incubated at 37 °C and 5% CO_2.

A 25-mM stock solution of chloroauric acid ($HAuCl_4$, Sigma) was prepared in phosphate buffered saline. The gold salt solution was sterilized by filtering through a syringe filter before adding to cells in the desired amount. Imaging was carried out with a Zeiss LSM 700 fluorescent confocal microscope equipped with a 10× objective. Data were quantified in Zeiss ZEN Black software.

Cell viability at 24 h after gold salt addition was evaluated using an MTS cell viability assay from Promega. Cells were grown to approximately 80% confluence in a 24-well plate. Stock gold salt solution was added to each well according to the desired gold salt concentration. The MTS assay was carried out at 24 h after addition of the gold salt. Cell absorbance measurements were collected using a plate reader set to 490 nm (Biotek).

3. Results and Discussion

The NG 108-15 cells were found to synthesize AuNCs intracellularly over a period of 24 h. This was confirmed via fluorescent confocal microscopy. Phase contrast and fluorescent micrographs for each gold salt concentration (0.1–10 mM) are shown in Fig. 1. Higher concentrations of salt solution led to an increase in fluorescence but did not appear to affect cellular morphology. There is evidence of similar amounts of cell spreading at all concentrations. A 100-mM gold salt concentration was attempted but immediately led to cell death and no data are included in this report. Quantification of the fluorescence data confirms that there is a significant increase in fluorescence intensity with increasing concentration of gold salt in the media for all concentrations (except between 0 and 0.1 mM) (Fig. 2). This is attributed to production of AuNCs within the cells.

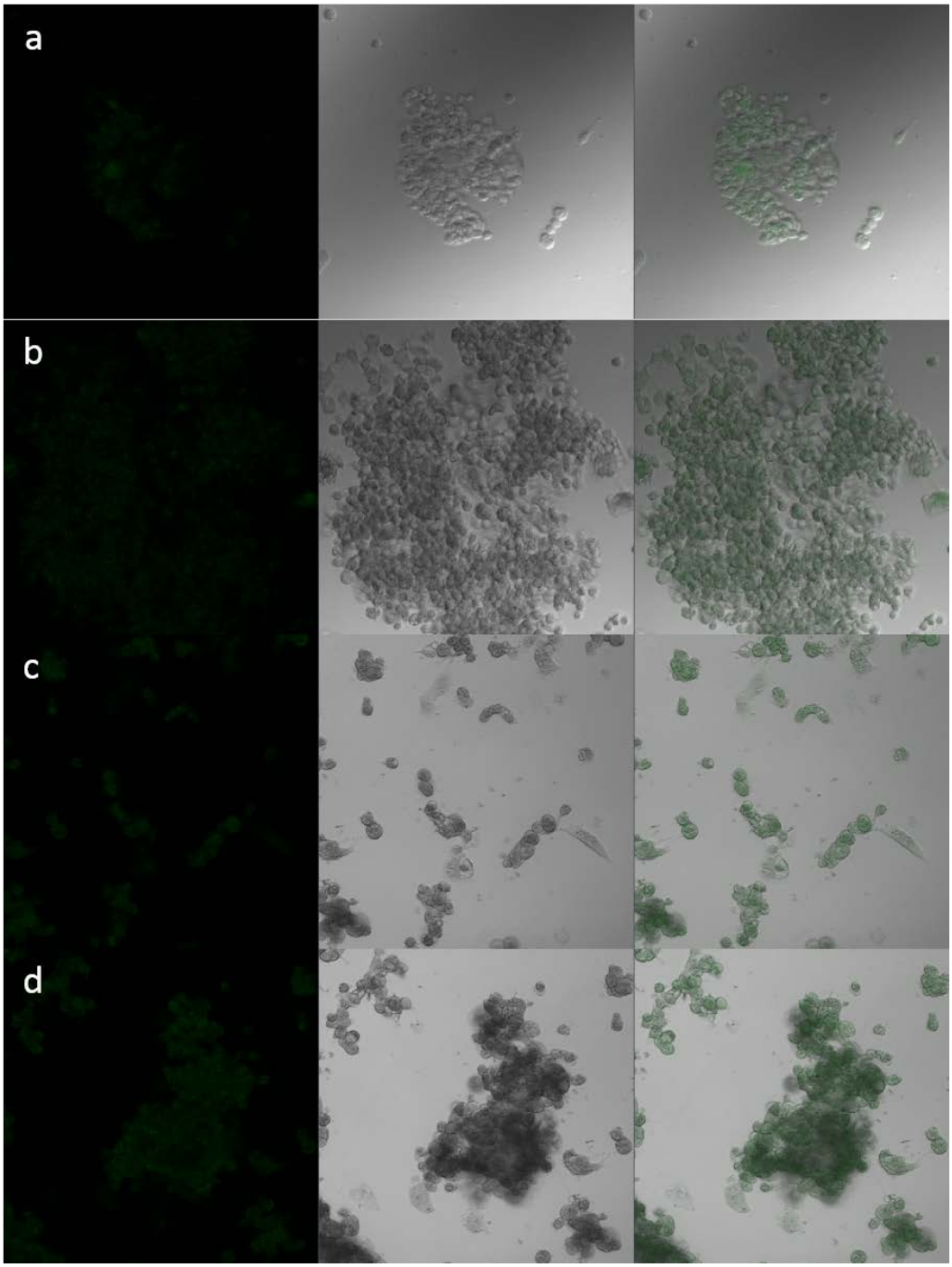

Fig. 1 Fluorescence, phase contrast, and combined confocal micrographs of NG108-15 cells 24 h after addition of a) 0 mM, b) 0.1 mM, c) 1 mM, and d) 10 mM of gold salt

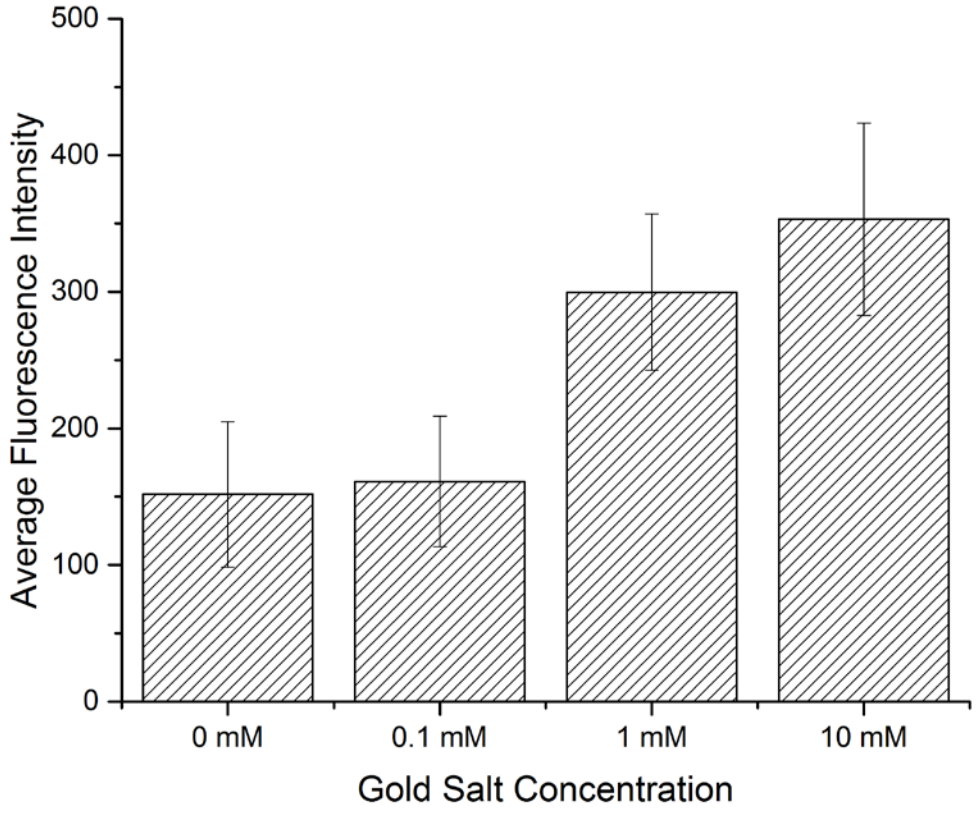

Fig. 2 Fluorescence intensity data from NG108-15 cells incubated with gold salt for 24 h

An MTS cell viability assay was performed on the cells to test viability in the presence of the gold salt. The MTS dye, in the presence of living cells, is reduced to a purple formazan product with an absorption maximum at about 490 nm. The viability was reduced at all concentrations of gold salt, compared with the control, but there was no significant difference in cell death between the concentrations evaluated (Fig. 3). The evaluation of cell death at time points beyond 24 h might yield different results, as it is expected that higher concentrations may be more toxic to the cells based on the results from the 100-mM solutions discussed previously. Potentially, other cell lines might yield brighter clusters with less cell death.

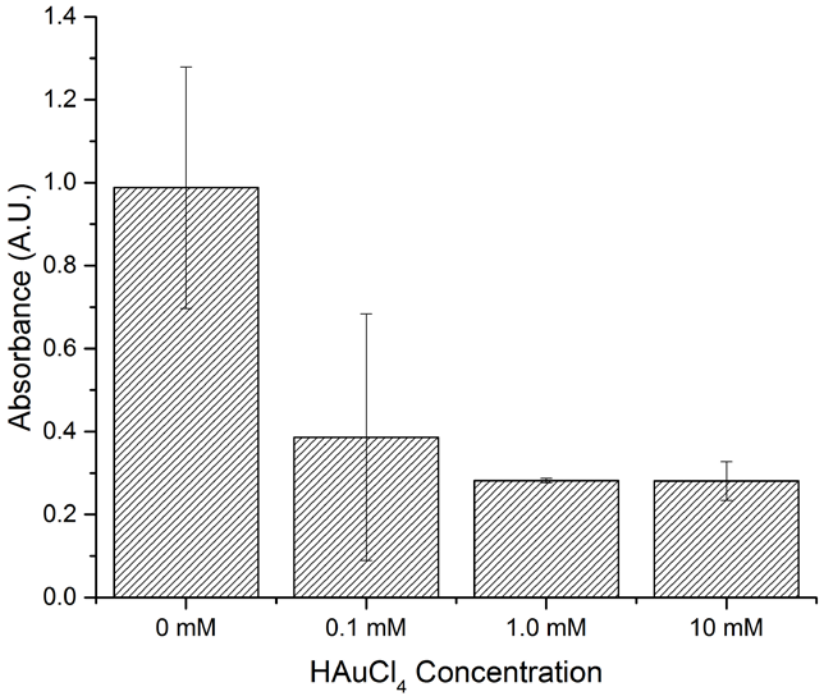

Fig. 3 Viability as determined by a MTS assay of NG108-15 cells incubated with varied concentrations of $HAuCl_4$ salt

4. Conclusions

It was shown that NG108-15 cells synthesize AuNCs when incubated in the presence of a gold salt. Since AuNCs are sensitive to pressure, this shows promise as a method to study the effects of blast trauma on neuronal cells. There is a decrease in cell viability as concentration of gold salt increases, but approximately 25% of the seeded cells remain viable even at concentrations as high as 10 mM.

5. References

1. Xavier PL, Chaudhari K, Baksi A, Pradeep T. Protein-protected luminescent noble metal quantum clusters: an emerging trend in atomic cluster nanoscience. Nano Rev. 2012;3:14767−14783.

2. Maity P, Xie S, Yamauchiab M, Tsukuda T. Stabilized gold clusters: from isolation toward controlled synthesis. Nanoscale. 2012;4:4027−4037.

3. Xie J, Zheng Y, Ying JY. Protein-directed synthesis of highly fluorescent gold nanoclusters. J Am Chem Soc. 2009;131(3):888−889.

4. Zhang M, Dang Y-Q, Liu T-Y, Li H-W, Wu Y, Li Q, Wang K, Zou BJ. Pressure-induced fluorescence enhancement of the BSA-protected gold nanoclusters and the corresponding conformational changes of protein. Phys Chem. 2013;117(1):639–647.

5. Kane MJ, Hatic H, Delic V, Dennis JS, Butler CL, Saykally JN, Citron BA. Modeling the pathobiology of repetitive traumatic brain injury in immortalized neuronal cell lines. Brain Res. 2011;1425:123–131.

6. Meaney DF, Smith DH. Biomechanics of concussion. Clin Sports Med. 2011;30(1):19–31.

7. Wang J, Zhang G, Li Q, Jiang H, Liu C, Amatore C, Wang X. In vivo self-bio-imaging of tumors through in situ biosynthesized fluorescent gold nanoclusters. Sci Rep. 2013;3:1157–1162.

1 (PDF)	DEFENSE TECHNICAL INFORMATION CTR DTIC OCA
2 (PDF)	DIRECTOR US ARMY RESEARCH LAB RDRL CIO LL IMAL HRA MAIL & RECORDS MGMT
1 (PDF)	GOVT PRINTG OFC A MALHOTRA
2 (PDF)	DIR USARL RDRL WMM G N ZANDER M GILLAN

INTENTIONALLY LEFT BLANK.